One Health approach to measure the impact on wellbeing of selected infectious diseases in humans and animals in Zambia

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Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy ©

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Declaration

I declare that the research described within this thesis is my own work and that this thesis is my own composition and certify that it has never been submitted for any other degree or professional qualification.

Kathrin Schaten

Edinburgh, 2017

Contents

Contents i

Tables x

Figures xiv

Text boxes xxi

Preface xxii

Dedication xxiii

Acknowledgements xxiv

Abbreviations xxvii

Abstract xxxi

Lay summary xxxiv

Chapter 1: General introduction 1

1.1 Introduction 1

1.2 How can we judge the wellbeing of a country and its people? 2

1.3 Wealth indicators 5

1.3.1 Macroeconomic wealth indicators in use 5

1.3.1.1 Gross domestic product (GDP) 5

1.3.1.2 Gross national income (GNI) 7

1.3.2 Alternative macroeconomic development indicators 7

1.3.2.1 Gini coefficient 7

1.3.2.2 Human development index (HDI) 8

1.3.2.3 Genuine progress indicator (GPI) or Index of sustainable economic welfare (ISEW) 8

1.3.2.4 Happy Planet Index (HPI) 8

1.3.2.5 Gross national happiness (GNH) 9

1.3.2.6 Organisation for Economic Cooperation and Development (OECD) better lives dashboard 9
1.3.2.7 Future orientation index 9
1.4 Human health 10
1.4.1 Universal health coverage (UHC) 10
1.4.2 The economics of human health 10
1.4.2.1 Disability adjusted life years (DALY) and other non-monetary measures of disease impact 11
1.4.2.2 Socio-economic status 13
1.4.3 Social determinants of health 13
1.4.4 Factors influencing the livelihood of people 14
1.4.4.1 Subjective wellbeing 14
1.4.4.2 Social contacts and network 15
1.4.4.3 Work situation 15
1.4.4.4 Health inequity and inequality 16
1.4.5 Neglected diseases of poverty 18
1.5 The role of livestock and animal health 23
1.5.1 Livestock and animal health economics 24
1.5.1.1 Gross margin analysis 24
1.5.1.2 Partial budget analysis 25
1.5.1.3 Herd Models 26
1.5.1.4 Value chains 26
1.5.1.5 Benefit-cost analysis 26
1.6 Environmental health and biodiversity 27
1.6.1 Ecosystem services 29
1.6.2 Role of wildlife in the disease cycle 30

1.6.3 Land use change ... 32

1.7 Holistic meaning of health - One Health ... 32

1.7.1 Historical development ... 33

1.7.2 The implementation of a One Health approach ... 33

1.8 Diseases of importance in Zambia ... 37

1.9 Aims of the thesis ... 37

1.9.1 Research questions ... 37

1.9.2 Work conducted ... 38

1.9.3 Work presented in this document ... 39

1.9.4 Software used ... 39

1.9.5 Ethical consent ... 40

1.9.6 Papers already published ... 40

Chapter 2: Infrastructure of the study area ... 43

2.1 Geography ... 43

2.2 Climate ... 48

2.3 Demography ... 49

2.3.1 Traditional societal system ... 49

2.3.2 Ethnicities ... 50

2.3.3 National census data ... 52

2.4 Flora and fauna in the valley ... 55

2.4.1 Wildlife ... 56

2.4.1.1 Aerial surveys and wildlife population trends in and around the South Luangwa National Park ... 57

2.4.2 Vegetation ... 58

2.5 School system ... 60

2.6 Health in Zambia ... 61

2.6.1 Health centres ... 61

2.7 Agriculture in the Eastern Province ... 62

2.7.1 Crop farming ... 64

2.7.1.1 Crop and vegetable production for food ... 64

2.7.1.2 Cotton production ... 66

2.7.2 Livestock production ... 67

2.7.2.1 Grazing and water ... 68

2.7.2.2 Animal production for food ... 69

2.8 Availability of veterinary services and drugs ... 72

2.9 Tourism ... 74

2.9.1 Conservation efforts ... 75

2.9.2 Hunting tourism, subsistence hunting and commercial poaching ... 76

Chapter 3: Survey on the disease status in animals ... 81

3.1 Introduction ... 81

3.1.1 Aims of the chapter ... 82

3.1.2 Trypanosomiasis in the study area ... 82

3.1.3 Tick-borne diseases in cattle and dogs in the study area ... 88

3.1.4 Brucellosis in the study area ... 91

3.1.5 Cysticercosis in the study area ... 94

3.1.6 African swine fever in the study area ... 97

3.2 Materials and Methods - Animals ... 98

3.2.1 Study design ... 98

3.2.2 Sample size calculation ... 98

3.2.3 Sampling protocol ... 101

3.2.4 Animal health questionnaire ... 101

3.2.5 Laboratory analysis ... 103

3.2.5.1 Sample taken ... 103

3.2.5.2 Sample preparation ... 106

3.2.5.3 Diagnostic techniques used for trypanosomiasis ... 107

3.2.5.4 Diagnostic techniques used for tick-borne diseases ... 112

3.2.5.5 Diagnostic techniques used for brucellosis ... 118

3.2.5.6 Diagnostic techniques used for cysticercosis ... 119

3.2.5.7 Diagnostic techniques used for African swine fever ... 120

3.3 Results ... 120

3.3.1 Questionnaire related information ... 121

3.3.1.1 Goats ... 122

3.3.1.2 Pigs ... 125

3.3.1.3 Dogs ... 128

3.3.1.4 Cattle ... 133

3.3.1.5 Sheep ... 140

3.3.2 Trypanosomiasis results ... 143

3.3.2.1 Trypanosomiasis in cattle ... 144

3.3.2.2 Trypanosomiasis in goats ... 147

3.3.2.3 Trypanosomiasis in sheep ... 149

3.3.2.4 Trypanosomiasis in pigs ... 150

3.3.2.5 Trypanosomiasis in dogs ... 152

3.3.3 Tick-borne disease results ... 155

3.3.3.1 Tick-borne microorganisms found in cattle ... 158

3.3.3.2 Tick-borne microorganisms found in dogs ... 161

3.3.3.3 Sequencing results ... 163

3.3.4 Multiple infections ... 164

3.3.5 Brucellosis ... 169

3.3.5.1 Goats 170

3.3.5.2 Cattle 171

3.3.5.3 Dogs 171

3.3.5.4 Sheep 171

3.3.6 Cysticercosis 171

3.3.7 African swine fever 172

3.4 Discussion 174

3.5 Conclusion 178

Chapter 4: Survey on disease status in people 181

4.1 Introduction 181

4.1.1 Aims of the chapter 181

4.1.2 Selected diseases reported from Zambia 182

4.1.3 Sleeping sickness in the study area 183

4.1.4 Malaria in the study area 189

4.1.5 Traditional beliefs 194

4.2 Materials and Methods humans 195

4.2.1 Study design humans 195

4.2.2 Sample size calculation humans 195

4.2.3 Sampling protocol humans 198

4.2.4 Human health questionnaire 198

4.2.5 Laboratory analysis 199

4.2.5.1 Taking the sample 200

4.2.5.2 DNA extraction in the lab 200

4.2.5.3 Confirmation of DNA integrity 201

4.2.5.4 Diagnostic techniques trypanosomiasis 202

4.2.5.5 Diagnostic technique malaria 206

4.2.5.6 Sequencing 207

4.3 Results 207

4.3.1 Questionnaire related health status 209

4.3.1.1 Maternal and reproductive health 210

4.3.1.2 Symptoms and signs 211

4.3.1.3 Prevention and treatments taken 211

4.3.1.4 Drug (ab) use 212

4.3.2 Sleeping sickness prevalence 212

4.3.3 Malaria prevalence 214

4.3.4 Tick-borne disease prevalence 221

4.4 Discussion 221

4.5 Conclusion 225

Chapter 5: Factors influencing well-being 227

5.1 Introduction 227

5.1.1 Aims of the study 227

5.2 Materials and Methods 228

5.2.1 Study design 228

5.2.2 Questionnaire 229

5.2.3 Analysis of the answers 231

5.3 General observations and perceptions 231

5.3.1 Poverty in the study area 231

5.3.2 Livelihood strategies in the area 232

5.4 Wellbeing in Zambia 233

5.4.1 Human wildlife conflict 233

5.5 Results from the questionnaire 234

5.5.1 Demography 236

5.5.2 Education ... 239
5.5.3 Risk factors human ... 239
5.5.3.1 Water and sanitation ... 240
5.5.3.2 Risk factors for selected diseases ... 244
5.5.4 Risk factors for animals ... 246
5.5.5 Wellbeing ... 250
5.5.5.1 Decision power ... 253
5.5.6 Poverty and socio-economic considerations ... 254
5.5.6.1 Goods and values ... 256
5.5.6.2 Resilience ... 260
5.5.7 Infrastructure of health system ... 263
5.5.7.1 Accessibility ... 263
5.5.7.2 Satisfaction ... 264
5.5.7.3 Service provision ... 264
5.5.8 Livestock keeping ... 264
5.5.8.1 Livestock population dynamics ... 266
5.5.9 Animal traction ... 269
5.5.10 Infrastructure of veterinary services ... 270
5.5.10.1 Accessibility ... 271
5.5.10.2 Satisfaction ... 271
5.5.10.3 Service provision ... 272
5.5.11 Crop and vegetable production ... 273
5.5.12 Infrastructure of environment and wildlife observed ... 278
5.6 Discussion ... 282
5.7 Conclusion ... 289
Chapter 6: General discussion ... 291

6.1 Summary of work undertaken in this thesis 291

6.2 Interpreting the results with a One Health perspective 292

6.2.1 Ecological changes and health 293

6.2.2 Wildlife and its role for generating an income.................................. 294

6.2.3 Crop production and its influence on health and poverty 295

6.2.4 Livestock production and its role for generating an income.............. 296

6.2.5 Animal health and human health systems... 297

6.2.6 Ecology and poverty 299

6.2.7 Social factors with impact on wellbeing 299

6.3 Recommendations and future work 300

Appendices 301

Appendix I Human health questionnaire 302

Appendix II Animal health questionnaire 306

Appendix III Poverty and health questionnaire 308

References 322

Tables

Table 1-1 Neglected tropical diseases (NTD) (WHO (NTD)) 19

Table 1-2 Neglected zoonotic diseases (NZD) (WHO (NZD)) 22

Table 2-1 National census population data from 1990-2010 (Central Statistical Office Zambia, 2003; Central Statistical Office Zambia, 2011; Central Statistical Office Zambia, 2012a; Central Statistical Office Zambia, 2012b) 54

Table 3-1 Household distribution of survey census 2012 98

Table 3-2 Sample size calculation for cluster sampling based on trypanosomiasis in animals; the rate of homogeneity is taken from trypanosomiasis in cattle in Nigeria 100

Table 3-3 Number of animals per species to be sampled in above specified number of clusters 101

Table 3-4 ITS PCR master mix preparation using Biotaq Red DNA polymerase for a total of 25 µl per sample (adapted from (Njiru *et al.*, 2005)) 108

Table 3-5 ITS PCR master mix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Njiru *et al.*, 2005)) 108

Table 3-6 Primer sequences used for ITS PCR (Njiru *et al.*, 2005) 109

Table 3-7 ITS cycling conditions (adapted from (Njiru *et al.*, 2005)) 109

Table 3-8 *Trypanosoma godfreyi* master mix preparation for a total of 25 µl per sample (adapted from (Masiga *et al.*, 1996)) 110

Table 3-9 Primer sequences used for Trypanosoma godfreyi (McNamara *et al.*, 1994) 110

Table 3-10 *Trypanosoma godfreyi* cycling programming (adapted from (McNamara *et al.*, 1994)) 110

Table 3-11 RLB PCR mastermix preparation for a total of 25 µl per sample (Lorusso, 2014) 113

Table 3-12 Primers used for the RLB protocol (Lorusso, 2014) 113

Table 3-13 RLB PCR cycling programming (Lorusso, 2014) 114

Table 3-14 Sequences of probes that were used on the RLB membrane (adapted from (Lorusso, 2014)) 115

Table 3-15 Goats life stage demography based on AHQ (entire means confirmed non-castrated) 122

Table 3-16 Pig life stage demography based on AHQ 125

Table 3-17 Dog life stage demography based on AHQ 128

Table 3-18 Cattle life stage demography based on AHQ 133

Table 3-19 Sheep life stage demography based on AHQ 140

Table 3-20 Tick-borne infections found in cattle; negative lower confidence interval limits were corrected to 0 159

Table 3-21 Tick-borne infections found in dogs, negative lower confidence interval limits were corrected to 0 162

Table 3-22 Multiple infections in goats 164

Table 3-23 Multiple trypanosome infection in cattle 165

Table 3-24 Multiple trypanosome infection in dogs 165

Table 3-25 Multiple tick-borne infections in dogs 166

Table 3-26 Multiple trypanosome and tick-borne infections in dogs 167

Table 3-27 Most frequent multiple trypanosome infection and TBI in cattle (n=53) 168

Table 4-1 Sample size calculation for cluster sampling in humans based on malaria 197

Table 4-2 Cytochrome b mastermix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Kocher *et al.*, 1989; Muturi *et al.*, 2011) 201

Table 4-3 Primer sequence used for cytochrome b PCR (Muturi *et al.*, 2011) 202

Table 4-4 Cytochrome b cycling conditions 202

Table 4-5 *T. brucei s.l.* PCR master mix preparation using Biotaq Red DNA polymerase for a total of 25 µl per sample (adapted from (Moser *et al.*, 1989) 203

Table 4-6 *T. brucei s.l.* PCR master mix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Moser *et al.*, 1989)) 204

Table 4-7 Primer sequence used for *T. brucei s.l.* PCR (Moser *et al.*, 1989) 204

Table 4-8 *T. brucei s.l.* cycling conditions (adapted from (Moser *et al.*, 1989)) 204

Table 4-9 SRA PLC master mix preparation (adapted from (Picozzi *et al.*, 2008)) 205

Table 4-10 Primer sequences used for multiplex PCR (Picozzi *et al.*, 2008) 205

Table 4-11 Multiplex PCR cycle programming (Picozzi *et al.*, 2008) 206

Table 5-1 Life satisfaction in the study area 250

Table 5-2 Changes that are needed to improve people's livelihoods and well-being 253

Table 5-3 Ranking minor investments people would undertake with improved income 255

Table 5-4 Energy sources used for lighting 256

Table 5-5 Energy sources used for cooking 257

Table 5-6 Energy sources used for heating 257

Table 5-7 Ownership of selected household assets in the study area 260

Table 5-8 Distribution of animal species across households based on household questionnaire 266

Table 5-9 Productivity calculations for animals 267

Table 5-10 Crops commonly grown in the area.. 274

Table 5-11 Frequency of contracts with cotton companies of the study area 278

Table 5-12 Vegetation changes perceived by the households over the past 5 years 279

Table 5-13 List of wildlife species seen by the households.................................... 280

Figures

Figure 1-1 Underlying definition of wellbeing in this thesis 4

Figure 1-2 Theory of the relationship between holistic meaning of health, poverty and wellbeing 4

Figure 1-3 GDP from UK, Zambia and South Africa from 2002-2016 as a comparison 7

Figure 1-4 An illustration of equity and equality (Valbrun, 2017) 17

Figure 1-5 Global overlap of six of the common NTDs (CDC, 2011) 20

Figure 1-6 A cattle kraal and a goat house on the left are meant to protect livestock from wild animal attacks 31

Figure 1-7 Relationship between detection of a zoonotic disease and costs related to time of detection taken from (World Bank, 2012) 35

Figure 2-1 Map of Zambia, showing the location of the study area 43

Figure 2-2 The GreatEast road on the plateau linking the border of Malawi via Chipata, Katete, Nyimba to Lusaka 44

Figure 2-3 Most roads in the valley are in poor condition 45

Figure 2-4 Map with households included in the survey 46

Figure 2-5 A bike is the most important mode of transport in the area (© J. Kuleszo) 47

Figure 2-6 When the car is stuck in the dry riverbed, everybody has to help (© J.Kuleszo) 47

Figure 2-7 Geographical distribution of chiefdoms in the Luangwa valley (Dalal-Clayton and Child, 2003) 50

Figure 2-8 Entrance to the South Luangwa national park 55

Figure 2-9 The Thornicroft giraffe is unique to the Luangwa valley (© J. Kuleszo) 56

Figure 2-10 A dried out riverbed is a good place to observe different species of wildlife (©J.Kuleszo) 58

Figure 2-11 Mopane woodland (© J.Kuleszo) 59

Figure 2-12 Schools in the area (GPS points taken during fieldwork therefore no claim to be complete) 60

Figure 2-13 Health centres in the area 62

Figure 2-14 Slash-and-burn agriculture (© J. Kuleszo) 63

Figure 2-15 A former wood cleared for farming (© J.Kuleszo) 64

Figure 2-16 Pack of rice produced by COMACO 65

Figure 2-17 A cotton field ready to harvest in April 67

Figure 2-18 Guinea fowls are the most valuable poultry kept in the area 68

Figure 2-19 A trader who transported pork on his bike to sell it in the area 71

Figure 2-20 Cattle head hanging in front of a shop in Mfuwe 72

Figure 2-21 Animal health related GPS points within the defined study area 73

Figure 3-1 Relict of the historical importance of trypanosomiasis in the area 84

Figure 3-2 Dip tank in the area 85

Figure 3-3 A typical model of a trap to catch tsetse flies 87

Figure 3-4 Life cycle of *Taenia solium* (© CDC) 95

Figure 3-5 The questionnaire was recorded into a tablet by a second person while the animal was sampled 102

Figure 3-6 Blood for the FTA card was taken with the help of a micro capillary from the ear vein 104

Figure 3-7 The blood sample was transferred to a FTA card using a microcapillary tube 105

Figure 3-8 For brucellosis testing blood was taken from the jugular vein (© N.Anderson) 106

Figure 3-9 A typical image of an agarose gel with results from an ITS PCR 111

Figure 3-10 Distribution of animal health questionnaires conducted (n=1226) 122

Figure 3-11 Body condition score of goats sampled (L=lean, M=medium, F=fat) . 123

Figure 3-12 Bad claw management in a goat (picture taken from animal health questionnaire) 124

Figure 3-13 Body condition score pigs 126

Figure 3-14 A pig with white lice (picture taken from animal health questionnaire) 127

Figure 3-15 Body condition score dogs 129

Figure 3-16 A puppy with mange and emaciation (picture taken from animal health questionnaire) 130

Figure 3-17 Myasis on the chin of a puppy (picture from animal health questionnaire) 131

Figure 3-18 Transmissible venereal tumour in a bitch (picture from animal health questionnaire) 132

Figure 3-19 Body condition score cattle 134

Figure 3-20 Downer cow (picture from animal health questionnaire) 135

Figure 3-21 Skin lesions in a cow (picture from animal health questionnaire) 136

Figure 3-22 Skin rush in a cow (picture from animal health questionnaire) 137

Figure 3-23 Fracture in a cow (picture from animal health questionnaire) 138

Figure 3-24 Stunted growth (picture from animal health questionnaire)................ 139

Figure 3-25 Body condition score sheep.. 141

Figure 3-26 Eye problem in a sheep (picture taken from animal health questionnaire) 142

Figure 3-27 Number of samples tested for trypanosomiasis (n=1275)................... 143

Figure 3-28 Cattle population sampled (n=340) 144

Figure 3-29 Distribution of cattle keeping households testing positive and negative for trypanosomiasis in cattle 145

Figure 3-30 Goat population sampled (n=473)... 147

Figure 3-31 Distribution of goat-keeping households testing positive and negative for trypanosomiasis in goats 148

Figure 3-32 Sheep population sampled (n=22)... 149

Figure 3-33 Pig population sampled (n=245) 150

Figure 3-34 Distribution of pig-keeping households testing positive and negative for trypanosomiasis in pigs.. 151

Figure 3-35 Dog population sampled (n=195).. 152

Figure 3-36 Distribution of dog-keeping households testing positive and negative for trypanosomiasis in dogs... 153

Figure 3-37 Emaciation can be a sign of trypanosomiasis...................................... 154

Figure 3-38 Samples tested for tick-borne diseases (n=535).................................. 155

Figure 3-39 Result sheet of an RLB 156

Figure 3-40 Cow with ticks marked by green arrows (taken from animal health questionnaire) 157

Figure 3-41 Distribution of cattle-keeping households testing positive and negative for TBI in cattle 158

Figure 3-42 Distribution of dog-keeping households testing positive and negative for TBI in dogs 161

Figure 3-43 Samples tested for brucellosis (n=750) 169

Figure 3-44 Map with the households tested for brucellosis 170

Figure 3-45 Map of households with pigs that tested positive for cysticercosis...... 172

Figure 3-46 Map of households with pigs tested for African swine fever 173

Figure 4-1 Natural vegetation in the valley with grass, bushes and trees; an ideal habitat for tsetse flies 184

Figure 4-2 Winterbottom's sign: Swollen posterior cervical lymphnodes (Van den Enden) 187

Figure 4-3 Malaria cycle (CDC, 2016) 190

Figure 4-4 Human health questionnaires were conducted away from others 199

Figure 4-5 Blood of a participant is brought onto a FTA card 200

Figure 4-6 Interpretation of results CareStart Malaria Test 207

Figure 4-7 Children were always happy to be in the focus of a camera; the girl on the left with the blue shirt shows the big belly that was observed often in children in the area 208

Figure 4-8 Human health questionnaires conducted (n=943) 209

Figure 4-9 Human population tested for sleeping sickness (n=1012) 213

Figure 4-10 People tested for sleeping sickness 214

Figure 4-11 Distribution of people tested for malaria.. 215

Figure 4-12 People tested for malaria by age and sex (n=1005) 216

Figure 4-13 Households with malaria cases 217

Figure 4-14 Number of people who tested positive for malaria per household....... 218

Figure 4-15 Another way of using a mosquito net to cover a puppy shed 219

Figure 4-16 Prevalence within the age group 220

Figure 4-17 Distribution of age in all positive cases 220

Figure 5-1 For the household questionnaire, either the household head or his wife were interviewed 230

Figure 5-2 Fence around a field to keep wild animals away.................................. 234

Figure 5-3 Tribe distribution in sampled households 237

Figure 5-4 Push and pull factors for migration 238

Figure 5-5 Dogs and chickens ‘cleaning’ the dishes- a possible source of sharing diseases... 240

Figure 5-6 One way of getting water in the dry season is to dig holes into the dried river beds... 241

Figure 5-7 Building a well is a dangerous task 242

Figure 5-8 A safer way of getting water is a borehole 243

Figure 5-9 Number of households who observed tsetse flies 244

Figure 5-10 Observation of tsetse flies by households 245

Figure 5-11 A bitch looking for leftovers to feed her puppies 247

Figure 5-12 Average of overall life satisfaction per household 251

Figure 5-13 Beautiful house with flowers on the roof 252

Figure 5-14 Quite a modern way of housing with solar panels, a satellite dish and a canvas cover to make the roof waterproof 258

Figure 5-15 A fresh built brick house with roof and door still to come 259

Figure 5-16 A typical bath or toilet hut 259

Figure 5-17 Mode of transport to the nearest health centre 263

Figure 5-18 Map of animal keeping households compared to non-animal keeping households 265

Figure 5-19 To get the cattle used to walking and working together their owners let them walk around with the harness 269

Figure 5-20 One way of using animal traction in the area 270

Figure 5-21 Cotton bags waiting to be picked up by the company 273

Figure 5-22 A woman separating the corn from the cob to store it later in the big bags: the number of bags suggests that this is a very wealthy household 275

Figure 5-23 Application pattern of pesticides in cotton growing households 276

Figure 5-24 Cotton work distribution amongst household members 277

Figure 5-25 Species cited as most often encountering wildlife 281

Text boxes

The text boxes in this document provide information that was mainly gained through observation or personal communication. Their intention is to enable a better understanding of what is going on in the study area.

Text box 2-1 Chief Tindi and the Chewas 52

Text box 2-2 How to catch a rat 70

Text box 4-1 Sleeping sickness case in St Francis hospital in Katete 189

Text box 5-1 Translations of answers in the questionnaire 236

Text box 5-2 Role of dogs in the study area 249

Text box 5-3 The blind woman 262

Preface

'I do not believe the emotional motivation behind my research invalidates my findings. On the contrary, I believe no intellectual work is of value unless there is deep personal and emotional involvement.'

Stuart A. Marks

Dedication

I dedicate this work to Dr Joseph Mubanga and Mr John Silutongwe.

Dr Joseph Mubanga played a very decisive although hidden role in the conduct of my thesis. His survey dating back to 2007 was the basis of my work. He facilitated my fieldwork and nourished the interest in the area.

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"Nyala ni mozi!"

Abbreviations

A.	Anaplasma
AAT	Animal African trypanosomiasis
ACT	artemisinin-based combination therapy
ADRA	Adventist development & relief agency
AHQ	Animal health questionnaire
AIDS	Acquired immune deficiency syndrome
AMR	Antimicrobial resistance
ART	anti-retroviral treatment
ASF	African swine fever
B.	Babesia
Br.	Brucella
BTL	bilateral tubal ligation
CBD	convention on biological diversity
CI	confidence interval
COMACO	Community Markets for Conservation
CPE	Certificate of primary education
CRB	community resources board
CSA	census supervisory area
CSO	Central Statistical Office
CSOM	chronic suppurative otitis media
DALE	disability adjusted life expectancy
DALY	disability adjusted life year
DE	design effect
df	de facto
dj	de jure
E.	Ehrlichia
ECF	East Coast fever
EP	Eastern Province
ESPEN	expanded special project for elimination of neglected tropical diseases

EWI	ecosystem wellbeing index
f	female
FAO	Food and Agriculture Organisation
FGD	focus group discussion
GBD	global burden of disease
GDP	gross domestic product
GMA	game management area
HAT	Human African trypanosomiasis
HDI	human development index
HeaLY	healthy life year
HHQ	Human health questionnaire
HIV	Human immunodeficiency virus
HWC	Human wildlife conflict
HWI	Human wellbeing index
IAEA	International Atomic Energy Agency
IO	international organisation
IRS	indoor residual spraying
ITN	insecticide treated net
ITS	internal transcribed spacer
KII	key informant interviews
LIRDP	Luangwa Integrated Resource Development Project
LLINs	long-lasting insecticidal nets
LPT	low pathogenic *Theileria*
LSA	Luangwa safari association
m	male
MCA	multiple correspondence analysis
NCC	neurocysticercosis
NGO	non-governmental organisation
NIP	neglected infections of poverty
NLNP	North Luangwa National Park
NS	nodding syndrome

NTD	neglected tropical diseases
NZD	neglected zoonotic diseases
OIE	World Organisation for Animal Health
P.	Plasmodium
PCR	Polymerase chain reaction
QALY	quality adjusted life year
R.	Rickettsia
RBT	Rose Bengal test
RDT	rapid diagnostic test
RLB	reverse line blot
SEA	standard enumeration area
SEM	structural equation modeling
s.l.	sensu latu
SLCS	South Luangwa Conservation Society
SLNP	South Luangwa National Park
sp.	Species
spp.	Species (Pural)
ssp.	Subspecies
STI	sexually transmitted infections
T.	Trypanosoma
TB	tuberculosis
TBD	tick-borne disease
TBI	tick-borne infection
TBP	tick-borne pathogens
Th.	Theileria
UHC	universal health coverage
UNICEF	United Nations International Children's Emergency Fund
UoE	University of Edinburgh
UNSIC	United Nations System Influenza Coordination
UNSTATS	United Nations Statistics Division

WASH	water, sanitation and hygiene
WCS	Wildlife conservation society
WHO	World Health Organisation
WQ	Wellbeing questionnaire
ZAWA	Zambian wildlife authority
ZMW	Zambian Kwacha

Abstract

This study describes the results of a cross-sectional survey conducted in Mambwe district in the Eastern Province in Zambia. It uses a One Health approach to assess the impact of veterinary, medical, environmental and social determinants on animal and human health and wellbeing. One Health is defined as a holistic and interdisciplinary approach that describes the complexities between people, animals, the environment and their health. Human wellbeing is defined in this thesis as *'a condition in which all members of society are able to determine and meet their needs and have a large range of choices to meet their potential' (Prescott-Allen, 2001)*. As a first step, eight focus group discussions with the inhabitants followed by key informant interviews with stakeholders in the area were conducted to give a primary impression and narrow down the problems in relation to animal and human health of the area in general.

Following this, a randomized selection of 210 households was visited and in each household blood samples were taken from all humans and all animals belonging to five animal species, namely cattle, goats, sheep, pigs and dogs. A third of the households did not keep any of the animal species chosen for sampling, but their inclusion was important for the social analysis. In all of these 210 households a wellbeing questionnaire was administered and, for every human and animal sampled, a health questionnaire.

The study area falls within the tsetse-infested region of Zambia. It has a high wildlife density reflecting the proximity of several national parks and is historically endemic for both human and animal African trypanosomiasis (HAT&AAT). Therefore humans and animals were tested for trypanosomiasis using internal transcribed spacer (ITS) polymerase chain reaction (PCR). Since it is important as a differential diagnosis, malaria was tested for by a rapid diagnostic test in the field from human blood. Sera from mature individuals from all animal species except pigs were tested in a field laboratory for brucellosis using the Rose Bengal test. Additionally, cattle and dogs were tested for five genera of tick-borne infections (TBI) including *Anaplasma*, *Ehrlichia*, *Theileria*, *Babesia* and *Rickettsia* using reverse line blot

(RLB) in the laboratory at the University of Edinburgh (UoE). The blood samples for PCR and RLB analysis at UoE were stored on Whatman™ FTA cards.

A total of 1012 human samples were tested for HAT and none found positive. 1005 (seven people had been tested positive or treated against malaria shortly before the sampling) people tested for malaria showed an overall prevalence of 15% (95% CI 13.2-17.7). None of the 734 Rose Bengal tests showed up positive for brucellosis. The prevalence of AAT in 1275 samples tested was much lower compared to former samplings; in cattle 22% (95% CI 18-27.2), in goats 7% (95% CI 4.5-9.2), in pigs 6% (95% CI 3.2-9.4), in dogs 9% (95% CI 5.2-13.6) and no samples were found positive in sheep. The prevalence of TBIs is much more complex with many multiple infections. A total of 340 cattle and 195 dogs were tested. In cattle the number of samples positive for any microorganism was as follows; 92% (95% CI 88-94.2). Overall there were fewer positive samples from dogs with 25% of animals infected (95% CI 19.2-31.8).

The wellbeing and health questionnaires were designed to help to identify possible risk factors for the above-mentioned diseases and signs, such as fever, diarrhoea and seizures, indicative for several other diseases. The results of these surveys might also help to identify potential reasons for a lower or higher prevalence of trypanosomiasis and malaria found than expected from previous studies. Additionally, information on personal happiness, attitudes towards veterinary and medical services, medical treatments received, education, women's reproductive history, drug abuse, people's perceptions of changes in environment and agriculture, demography, poverty and migration were collected via the questionnaires alongside information on livestock demographics and fertility.

One of the main conclusions is that both medical and veterinary health care systems suffer from a number of shortcomings. The distance to appropriate treatment and care facilities is far and the necessary drugs are often unavailable. Also, both the knowledge and technology for diagnosing selected diseases is not in place. This study suggests that neurocysticercosis (NCC) plays an important role in this area due to the high number of seizures reported in people, in whom treatment for epilepsy was unsuccessful. Samples taken from a few pigs indicated the presence of *Taenia* *solium*, the causal agent of NCC. Furthermore, many of the TBIs are of zoonotic nature and further investigations must be made to begin to assess the burden of these diseases in humans and animals. Environmental changes such as degradation of the vegetation are likely to have an influence on the prevalence of studied diseases and this aspect is being investigated further in other studies. Due to the nature of a cross-sectional study, only limited conclusions can be drawn on the causal relationships of disease prevalence, but the social analysis conducted in this study confirmed the interactions of selected factors related to health and wealth unique for this study area.

PRESCOTT-ALLEN, R. (2001) *The wellbeing of nations*, Island Press

Lay summary

In most countries of the world, there is thought to be an association between people's health and their wealth. Only people who have access to health information are able to make sound judgements about their health status and related risks; people who live in countries with a good health system and who can afford any prevention or treatment needed will be more likely to have a higher level of wellbeing. These are the hypotheses that drive the studies described in this thesis. Human wellbeing is defined here as 'a condition in which all members of society are able to determine and meet their needs and have a large range of choices to meet their potential' (Prescott-Allen, 2001).The study area is in the Eastern province in Zambia, an area with touristic activity due to its proximity to several national parks and where most of the inhabitants are smallholder livestock farmers. Literature suggests that keeping livestock, notably cattle, is a sign of wealth in many countries in Africa. This study attempted to find a more broadly based composite indicator of poverty so as to assign the 210 households studied to a certain wealth level. The health status of people and their animals was assessed, by sampling cattle, goats, sheep, pigs and dogs, emphasising the diagnosis of diseases that are shared between humans and animals, the so called zoonoses. Discussions and interviews were conducted to obtain a more comprehensive overview of the problems encountered in the area and their influence on health, poverty and wellbeing.

The main problem found was that the health care systems for humans and animals are not functioning particularly well. The distance to the facilities is far and drugs are not always available when needed. Additionally, the pattern of disease presence has changed as new diseases have emerged and others play a less important role now. The health systems have not yet adapted to these changes. People are generally quite poor in the area if they are not employed and climatic conditions influence their harvest success every year. The education level is very low, with an illiteracy rate of around 70%. This study was able to identify some specific health-related problems that require further investigations so as to improve the wellbeing of the people in the Eastern province of Zambia.

Chapter 1: General introduction

The work presented within this thesis is part of the wider project of the Dynamic Drivers of Disease in Africa Consortium (DDDAC). The DDDAC project focused on four diseases in five African countries; Lassa fever in Sierra Leone, Henipavirus in Ghana, Rift Valley fever in Kenya and trypanosomiasis in Zambia and Zimbabwe.

My colleagues from the University of Southampton/ Lancaster (Prof. Peter Atkinson, Simon Alderton and Dr. Joanna Kuleszo) together with Dr Neil Anderson from Edinburgh are developing agent-based models of disease transmission and risk mapping approaches that incorporate movement information as well as landscape ecology concepts whereas I am focussing on the epidemiology of trypanosomiasis and related risk factors for this disease. My work also covers the epidemiology of selected other diseases of interest in the study area in humans and animals, their economic implications and the overall wellbeing of the people. I use a One Health approach to identify the factors that are associated with, and influence disease occurrence and control. The Zambian team are the late Dr Joseph Mubanga, Dr Noreen Machila and Dr Martin Simuunza. They facilitated and supported the field work. Team members based in Edinburgh are Prof. Sue Welburn and Prof. Mike Thrusfield, who both directed the research, and Dr Ewan MacLeod and Pauline McManus, who were in charge of the local project management. Advice on socio-economic and poverty-related aspects of the work was given by Alex Shaw.

Through the implementation of the project, the aim is to understand how changes in ecosystem may have had an influence on disease incidence and vice versa, and finally to understand local perceptions, responses and the impact on livelihoods. A follow-up study would make any causal relationships more evident.

1.1 Introduction

Some countries in the world are more fortunate than others. For decades, experts tried to find a quantifiable and objective measure to visualize these differences. The theory behind these measures was that a more fortunate country would also be wealthier. And when a country is wealthier, its population must have a better quality of life. Therefore the gross domestic product (GDP) was used to define the level of development of the country in international comparisons. However, using GDP has some weaknesses and other indices were created to replace or complement it. The following sections will present some examples and clarification on this issue.

1.2 How can we judge the wellbeing of a country and its people?

Wellbeing is something that is not clearly defined and that comprises both objective and subjective or emotional elements.

The King of Bhutan (Jigme Singye Wangchuck) initiated an international discussion on how to judge the development of a country (UN, 2011a; UN, 2011b). He did not want to look at economic development alone, but on *'development with values'*. His concept is the basis for gross national happiness (GNH) as an indicator as much as a philosophy.

Rath and Harter wrote a whole book about wellbeing, where wellbeing is 'about the combination of our love for what we do each day, the quality of our relationships, the security of our finances, the vibrancy of our physical health, and the pride we take in what we have contributed to our communities. Most importantly, it's about how these five elements interact' (Rath and Harter, 2010). Therefore they divide wellbeing into career wellbeing, social wellbeing, financial wellbeing, physical wellbeing and community wellbeing.

Another approach is the human wellbeing index (HWI) (Prescott-Allen, 2001). It comprises 10 elements, namely; health, population, household wealth, national wealth, knowledge, culture, freedom and governance, peace and order, household equity and gender equity (Prescott-Allen, 2001). In this context, the human development index (HDI) expresses the progress away from poverty and is based on three elements with four indicators: health (life expectancy), wealth (income) and knowledge (illiteracy and school enrolment) (Prescott-Allen, 2001). Each of the above mentioned elements for the HWI have their own indices. The results for Zambia are discussed in the wellbeing chapter.

Another important index to be mentioned here is the ecosystem wellbeing index (EWI) (Prescott-Allen, 2001). It consists of five dimensions with altogether ten elements; land diversity, land quality, inland waters, sea, global atmosphere, local air quality, wild diversity, domesticated diversity, energy and materials, and resource sectors (Prescott-Allen, 2001). Again, the results for Zambia are listed in the wellbeing chapter.

All above-mentioned definitions and indices make sense for measuring wellbeing. However, this thesis had to restrict its definition to what is possible to measure within a PhD project. Therefore the basic concept of the definition of wellbeing in this thesis is presented in Figure 1-1.

Figure 1-1 Underlying definition of wellbeing in this thesis

The underlying hypothesis for this thesis is represented in Figure 1-2. The arrows indicate what has an impact on what. As such, health status of people has an impact on their well-being, because if someone is ill, he is not feeling well (Christoph, 2010). But well-being can also have an impact on health as there are psychosomatic conditions that play a role for the immune system too. Health and poverty have an often discussed inter-relationship. Last but not least, poverty can have an impact on well-being since deprivation of certain services may inhibit full participation in everyday life (Christoph, 2010). Nevertheless, there are two theories that limit the interrelationship between poverty and wellbeing, the adaptation level theory and the aspiration level theory (D'Acci, 2011). The former stands for the speculation that happiness increases with the improvement of our conditions but this increase tapers off the richer we become. The latter describes the assumption that happiness depends on the gap between what we wish for and how much we have (D'Acci, 2011).

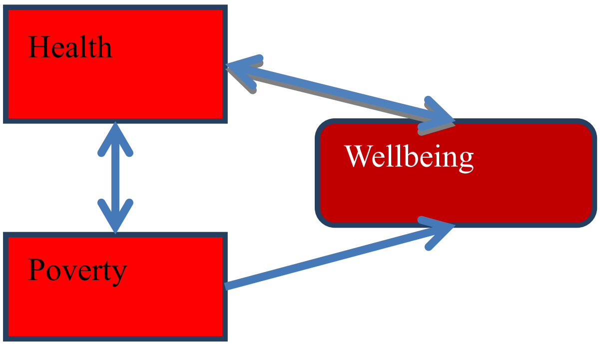


Figure 1-2 Theory of the relationship between holistic meaning of health, poverty and wellbeing

All these three factors are influenced or defined by many other things, such as the environment, keeping animals and their health status, access to health services, the infrastructure provided for income generation, and the power a person has to lead the life they want. With this, the study does not make a claim to be comprehensive. Human well-being is such a complex concept - this study's goal was to investigate what information could be obtained from visiting and interviewing in local households to gain an impression of some of the key components influencing human well-being in the study area.

1.3 Wealth indicators

Wealth describes the economic status of a country or an individual. The achievement of wealth differs depending on the social, environmental and political circumstances. Poverty, is a relative term, based on the comparison of a given income to the overall distribution of incomes in a population (Arcaya *et al.*, 2015; Citro and Michael, 1995).

In the following sections are examples of indicators that look at the economic or socio-economic status of nations. As the word already says, socioeconomics is a mixture of two disciplines, economics and sociology, and uniquely related to the culture, time and place where it is studied. Economics can again be divided into macro- and microeconomics, depending if we look at the economics of a country or an enterprise.

1.3.1 Macroeconomic wealth indicators in use

Macroeconomic wealth indicators are used to compare the economic performance of countries and are meant to give evidence about the level of development of the country and the wealth status of the inhabitants of this country.

1.3.1.1 Gross domestic product (GDP)

The gross domestic product (GDP) defines itself by the market value of all officially recognized final goods and services produced within a country in a given period. The period usually considered is one year. The GDP is commonly used as an indicator for the wealth of the citizens of a country, although it does not take the standard of living into account, because not everybody benefits equally from a country's economic output.

The use of the GDP as a macroeconomic indicator has some advantages. First, it is measured frequently and regularly. Furthermore, it allows inter-country comparisons, because it is consistently calculated worldwide.

There are however a number of disadvantages, because the GDP does not reflect inequality nor the real purchasing power of the inhabitants. More modern thinking criticizes the missing consideration of the impact on the environment and biodiversity in the country. Quite often increased industrial production has a negative impact on the ecosystem, because it is exploiting its resources or producing too much, or even toxic, waste. The GDP also does not fully reflect all types of work, e.g. subsistence farming or house work. And last but not least, it does not give evidence about how happy or satisfied people are to live in this country.

A comparison between the GDP of Zambia, South Africa and the UK can be seen in Figure 1-3.

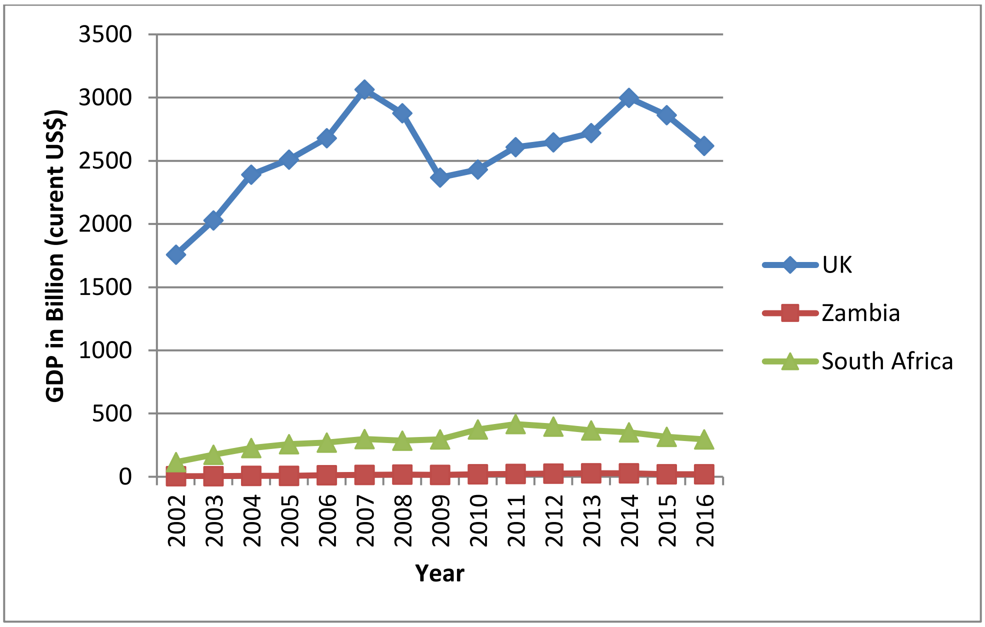


Figure 1-3 GDP from UK, Zambia and South Africa from 2002-2016 as a comparison

1.3.1.2 Gross national income (GNI)

The gross national income (GNI) sums up the value produced by all residents and the net receipts of primary income from abroad, gained through employment or property income (World Bank, 2012). In other words, the GNI is the GDP less primary incomes payable to non-resident units plus primary incomes receivable from non-resident units (OECD, 2012). In simple terms, GDP measures what is produced within a country's borders, where as GNI measures what is produced by its citizens.

1.3.2 Alternative macroeconomic development indicators

To improve the analysis and comparability of wealth/quality of life between different countries and within a country several alternative indicators have been developed, all focussing on different aspects of the socio-economic status or development.

The idea of mentioning them is to take account of the different aspects of wealth in society and to give credit to those aspects in the study on socio-economics in Zambia. Questions about the well-being of the people and the integrity of the environment in which they live could add valuable information.

1.3.2.1 Gini coefficient

The Gini coefficient reflects the inequality of the wealth and income distribution in a country (OECD, 2016). It is based on the Lorenz curve, which plots the cumulative shares of income earned against the cumulate of percentage of people, from the poorest to the richest (Lorenz, 1905). At theoretical extremes, a Gini coefficient of 0 stands for perfect equality, meaning everybody has the same income. In contrast, a Gini coefficient of 100 represents perfect inequality (where one person would receive all the income). As an example, the Gini coefficient of Zambia was 52.6 in 1998 and decreased to 50.8 in 2004 (OECD, 2016). This shows that the wealth distribution in the country has improved during this time period. As a comparison, the UK had a Gini coefficient of 36.8 in 1999 which improved to 34.0 in 2005 (OECD, 2016).

1.3.2.2 Human development index (HDI)

The HDI is a calculating method to compare the quality of life defined by life expectancy, literacy, education status, and standards of living in a country (UNDP, 2016). It is based on gross national income (GNI), life expectancy and the education level. It uses four categories to classify development: very high human development, high human development, medium human development, and low human development countries.

1.3.2.3 Genuine progress indicator (GPI) or Index of sustainable economic welfare (ISEW)

The genuine progress indicator (GPI), formerly known as the index of sustainable economic welfare (ISEW) and created in 1995, is a measure of economic performance and its sustainability for the environment of a country. It adds value to household and volunteer work and decreases value for crime (Redefining progress, 2016).

1.3.2.4 Happy Planet Index (HPI)

The HPI was created by the New Economics Foundation in 2006 and measures the ecological efficiency for achieving human well-being and reduced environmental impact. It is based on individual life satisfaction, life expectancy and the 'Ecological Footprint' (New Economics Foundation, 2016).

1.3.2.5 *Gross national happiness (GNH)*

The GNH was initiated by the King of Bhutan and presented on an UN conference on ‘Happiness and Well-being: Defining a New Economic Paradigm’. The GNH is a single number index based on 33 indicators categorized under nine domains. It measures a complex set of fields subjectively and objectively, such as living standard, health, education, ecosystem diversity and resilience, cultural vitality and diversity, time use and balance, good governance, community vitality and psychological well-being (Permanent Mission of the Kingdom of Bhutan, 2005).

1.3.2.6 *Organisation for Economic Cooperation and Development (OECD) better lives dashboard*

The Organisation for Economic Cooperation and Development (OECD) produced in 2011 a compendium of indicators that measure wellbeing and welfare outcomes (OECD, 2017). Some indicators used are real household disposable income, net cash transfers to households, real household consumption expenditure, consumer confidence, households’ savings rate, households’ indebtedness, financial net worth, unemployment rate, and labour underutilisation rate (OECD, 2017).

1.3.2.7 *Future orientation index*

The theory behind the future orientation index is that people coming from countries with a higher GDP are more probably searching on the internet for information on the future, using just Google, compared with people coming from countries with a lower GDP. Preis and others (Preis *et al.*, 2012) have observed this in their study. The number of queries per country altogether also gives a hint of the development of the country because it relates to the possibility of having internet access.

1.4 Human health

The definition of health from WHO is "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition." (WHO, 1948). With this definition, health is already very complex and difficult to measure. The following sections will try to highlight some aspects of health or factors influencing human health.

1.4.1 Universal health coverage (UHC)

Health system strengthening leads to universal health coverage. "UHC is based on the principle that all individuals and communities should have access to quality essential health services without suffering financial hardship. UHC cuts across all health targets and contributes to health security and equity." (WHO, 2017b).

According to the new sustainable development goals, UHC is the main aim for achieving good health in people (UN, 2015). UHC is based on three pillars; offering good quality of health care services, making health care affordable and providing access to health care for everybody.

1.4.2 The economics of human health

The economics of human health deals with the impact of ill health in people and the costs of mitigating those impacts. The central issue is the how to assess the value of human life and suffering. The main issue is whether to adopt monetary values or to use one of the various composite non-monetary measures, usually grouped under the concept health-adjusted life years (HALYs).

The literature on health economics discusses the pros and cons of assigning monetary values (following on from the original article by Mishan (1971) and summarised, for example in Fox-Rushby and Cairns, 2005) (Fox-Rushby and Cairns, 2005; Mishan, 1971). The main options for assigning monetary values have been the human capital approach (where people's lives are valued in relation to their contribution to the economy), studying observed, revealed or stated preferences (how much people are willing to invest in safety and other measures designed to prolong life or reduce risks, as implied by their behaviour or their statements about how much they would spend and other information such as awards for injuries and death and life insurance payments). The main disadvantage of these approaches is that the human capital approach implicitly places a higher value on the lives of wealthier people, and that the various preference measures suffer from biases in terms of people's appreciation of risk and their willingness to pay in order to avoid it.

1.4.2.1 Disability adjusted life years (DALY) and other non-monetary measures of disease impact

Thus the emerging consensus has been to adopt various HALYs. These measures have a long history and include healthy life year (HeaLY) (Weinstein and Stason, 1977). The quality adjusted life year (QALY), the disability-adjusted life expectancy (DALE). The QALY is a measure of utility and thus aim to quantify the quality of life (Whitehead and Ali, 2010). It has been widely used for many years, and is based on people's assessment of their quality of life, with standard questions and scoring bases being developed such as Euroqol (https://euroqol.org/). DALE incorporates the severity weights used in the global burden of disease study and mainly reports on the burden of disability and morbidity in non-fatal health outcomes (Murray and Lopez, 1997). The indicator HeaLY uses again a different calculation method for the estimation of morbidity and mortality (Hyder *et al.*, 1998).

The most recently developed HALY is the disability adjusted life year (DALY). Unlike the QALY, which measures quality of life, the DALY measures the burden of disease. Thus disease increases the number of DALYs whereas it reduces the number of QALYs. Using the DALY, in 1996, the first global burden of disease (GBD) study was published. It quantified mortality and morbidity for more than 100 diseases and injuries by age, sex and region. An update was published 2004 which included methodological innovations (Mathers, 2008). The burden of disease in humans is measured in disability adjusted life years (DALYs) which represent years of 'healthy' life lost for the whole population. DALYs for a disease or health condition are calculated as the sum of the years of life lost (YLL) due to premature mortality in the population and the years lost due to disability (YLD), where the duration of the illness is multiplied by an appropriate disability weight (Murray, 1994; WHO, 2012c).

As a clear and simple concept, applied on a global scale, the DALY has proved to be a powerful tool for assessing the impact of disease, comparing burdens across diseases, continents and wealth groups and prioritising resources for health interventions. Inevitably some weaknesses have been identified and critiqued. Murray and Chen argue that with decreasing mortality, morbidity or ill-health play an increasing role (Murray and Chen, 1992). However to assess that has been proven difficult, since for example people from the US report of more episodes of morbidity than people from poor regions in India (Murray and Chen, 1992). This has to do with the perceptions and expectations people have on health (Murray and Chen, 1992). The judgement is based on education, culture, income and the health system in the country. Other critiques have focussed on age-weighting whereby slightly lower weights are applied to the very young and the very old and to discounting (the process discussed above).

To answer some of these criticisms and refine and improve the DALY estimates, the Global Burden of Disease 2010 initiative was launched, involving a large cohort of experts internationally. A major emphasis was on reassessing disability weights. One objective was not to assign a single disability weight to each disease, but to analyse the various possible courses of the disease, to estimate their disability weight and to calculate the proportion of people affected and the appropriate weight applied. Face-to-face and online interviews were conducted using the method of called 'paired comparisons', where by people gave their opinions on the relative severity of two disease conditions. The key results of GBD2010 were initially published in a series of articles in The Lancet. Since then there have been a number of updates and further refinements, which are regularly made available at The Institute for Health Metrics and Evaluation website (http://www.healthdata.org/gbd) and in the Lancet (http://thelancet.com/gbd).

1.4.2.2 Socio-economic status

The socio-economic impact of a disease is culturally dependent, since the disease situation depends on the number and density of animals and humans that live together and on the environment surrounding both. Furthermore every culture has a different way of living with different wealth values and different ways of making money. The more this situation is known and considered in the calculation, the closer we come to the true socio-economic impact of a disease. It is also wise to assess several possible health outcomes, since the impact might vary a lot in different settings. For instance, Zika virus transmission may be linked to poverty since increased per capita GDP correlates with lower rates of Zika virus-linked microcephaly cases, a proxy for Zika virus incidence, in Brazil (Ali *et al.*, 2017).

The methods to assess the socio-economic status derive from sociology. They imply a lot of observation and questioning of the studied population.

1.4.3 Social determinants of health

The social determinants of health (SDH) are defined as "the conditions in which people are born, grow, work, live, and age, and the wider set of forces and systems shaping the conditions of daily life. These forces and systems include economic policies and systems, development agendas, social norms, social policies and political systems." (WHO (SDH)). They are related to the risk and probability of acquiring certain illnesses or injuries.

Graham again differentiates between social determinants of health and social determinants of health inequalities with the one common theme being social position (Graham, 2004). Social position is key to access to societal resources and therefore considered as fundamental over time and across all health outcomes (Graham, 2004). The social position may explain differences in behaviour and physiology, environmental risk and exposure and inequalities in illness and injury. So the social factors that have an impact on health are not the same as the social processes that frame their unequal social distribution (Graham, 2004).

Another important point raised by Carr *et al.* is that the perception of health and its meaning for one person or between several persons can be very different. Every person judges their health related quality of life as the difference between experience and expectations (Carr *et al.*, 2001). Therefore people with diverse expectations may report the same health condition differently (Carr *et al.*, 2001).

1.4.4 Factors influencing the livelihood of people

There are many factors that have been studied to play a role for health status, material status or wellbeing such as safety and security, freedom and rights, education and personal happiness. Some factors will be explained in more detail here.

1.4.4.1 Subjective wellbeing

Subjective wellbeing measures the self-reported feelings a person has (Tinkler and Hicks, 2011). Its subjectivity is inherent, as it relies on self-reporting and of feelings. Objective measures such as income, employment status, marital status, health and major life events do not take account of individuals' personal perceptions, and human perception is needed to understand the wellbeing of a person. But objective measures correlate well with subjective wellbeing measures (Dolan *et al.*, 2008; Tinkler and Hicks, 2011).

Objective measures of wellbeing either aim at objective list or preference satisfaction accounts (Tinkler and Hicks, 2011). Objective list accounts look if the basic pre-requisites are met before people can live better. Preference satisfaction accounts argue that the more people satisfy their preferences, the greater is their wellbeing. The policies coming out of these two concepts are very different, as one will try to improve objective circumstances such as education or health, whereas the other will raise people's income to increase choices (Tinkler and Hicks, 2011).

1.4.4.2 Social contacts and network

Human beings are biologically not meant to live alone. The number and strength of social relationships has a significant impact on mortality and longevity (Sagan, 1987). It does not depend if these relationships are through friendship or family (Sagan, 1987).

The digital development has globalised our network of friends, family, colleagues and business partners. This leads to increased travel activities all over the world, may it be for work or leisure. Important here is that 8% of travellers from industrialised to developing countries experience episodes of ill-health severe enough to seek medical help during or after their trip (Harvey *et al.*, 2013). This figure includes only travellers from the US between 1997-2011.

1.4.4.3 Work situation

The work situation is important for the livelihood of people because it exerts a major influence on the socioeconomic status of a person and her household. But work is not only related to economic considerations, but also to stress caused by work or by being jobless.

Although the biological interrelationship is not clear yet, it is well known that health depends on socioeconomic circumstances (Brunner, 1997). Studies in non-human primates have shown that the effects of dominance hierarchy on biology are similar in white collar civil servants. Psychosocial influences such as social position have a direct connection to stress and thus health inequalities (Brunner, 1997). Similarly, Cohen *et al.* investigated the role of social stress and social status on the susceptibility to upper respiratory infection in monkeys (Cohen *et al.*, 1997). As a result, low social status was related to a higher probability of infection (Cohen *et al.*, 1997).

A concept that is mostly discussed in developed countries recently is the basic income. People are given a moderate amount of money regardless if they work or not. In 2008-2009, the Namibia Basic Income Pilot Project was conducted to assess the impact of a basic income for the first time in a developing country. The results showed increased community mobilisation and empowerment, the poverty decreased significantly, increase in economic activity, reduction of child malnutrition, higher school attendance rates, reduction of household debts with increasing ownership of livestock, higher usage of local health infrastructure, significant reduction of crime, gender issues decreased and women empowerment (Basic Income Grant Coalition, 2009). There was a fear of increased alcoholism but data could not support that. Looking at the national financial capacity to finance a basic income on a long-term basis and provide sustainability to the project, were evaluated positively (Basic Income Grant Coalition, 2009; Suzuki, 2011). Nevertheless, there was also criticism on the methodology of the project and the interpretation of the results (Osterkamp, 2013). Beck *et al.* published a basic income study in India where the outcomes considered were minor illnesses and injuries, illness and injuries requiring hospitalisation, and child vaccination coverage (Beck *et al.*, 2015). The improvement especially on minor illnesses and injuries was high (Beck *et al.*, 2015).

1.4.4.4 Health inequity and inequality

Inequality refers to health differences that may be reduced but not eliminated; the differences may be due to genetics or aging. Inequity refers to differences that are unfair and preventable; action can be taken to reduce inequities. Inequities are not only existent in health but in many other sectors such as education, gender, economic etc. An important fact that WHO mentions is the accompanying lack of power:

"A characteristic common to groups that experience health inequities—such as poor or marginalized persons, racial and ethnic minorities, and women—is lack of political, social or economic power." (WHO, 2017a).

A nice illustration of equality, equity and justice can be seen in Figure 1-4.

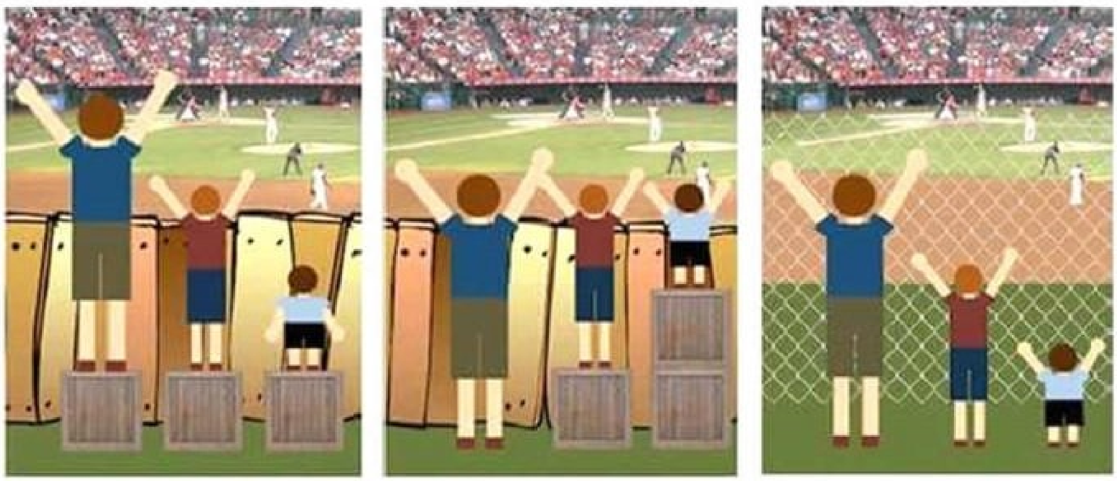


Figure 1-4 An illustration of equity and equality (Valbrun, 2017)

In the first image everyone receives the same support but they do not benefit in the same way. This represents equality. In the second picture, the individual person receives adapted support so that they can all watch the match. This represents equity. In the third image, all can watch the match without any support because the cause of the inequity has been removed. This situation represents justice.

Inequality in itself might cause ill-health. A study looking at income inequality and population health found that more people become ill in less equal societies, especially looking at large areas (Arcaya *et al.*, 2015; Wilkinson and Pickett, 2006).

Biggs *et al.* had similar results when they looked at the influence of national income level, poverty and inequality on public health in Latin America (Biggs *et al.*, 2010). As measurable figures they chose purchasing power parity, extreme poverty rates and the Gini coefficient. As public health outcomes they used life expectancy, infant mortality rates and tuberculosis (TB) mortality rates. They found out that poverty has an independent impact on the relationship between national income level and health, but the size of the impact depended on the inequality found in that country. So the aim should not be to increase the wealth of a nation but to distribute it more equitably in order to improve the health status of its people (Biggs *et al.*, 2010). As another example, Chowdhury *et al.* report on the exceptional health achievements in Bangladesh despite the economic poverty. The likely explanation given is the reduction in some inequities (Chowdhury *et al.*, 2013).

Some inequalities are biased by selection. It may not be that people are less active because they live in a less walkable area, but they have chosen to live there, because they do not like to be physically active (Arcaya *et al.*, 2015). So some inequalities may be based on self-selection and choice.

1.4.5 Neglected diseases of poverty

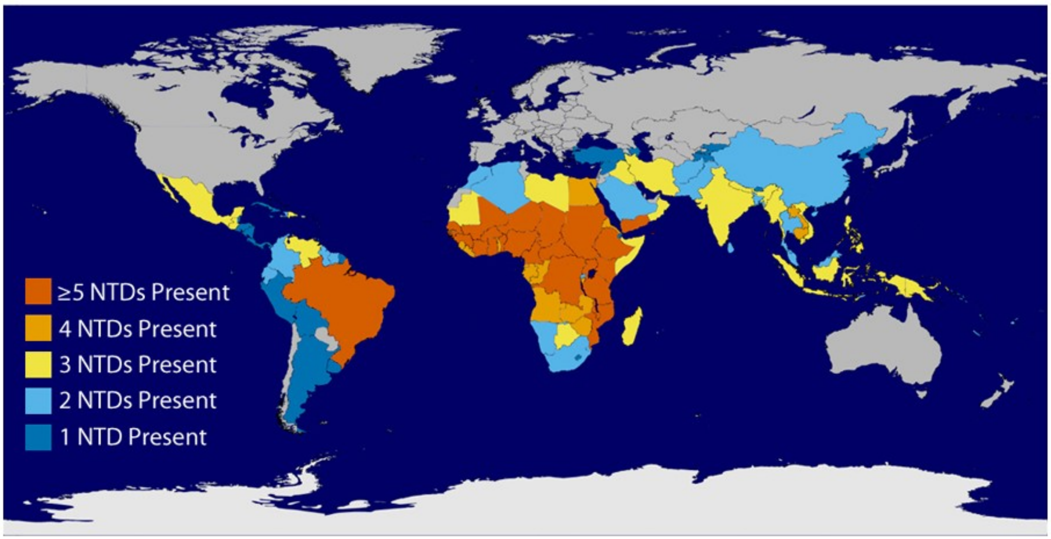
WHO defines neglected tropical diseases (NTDs) as "a diverse group of diseases with distinct characteristics that thrive mainly among the poorest populations. The 17 NTDs prioritized by WHO are endemic in 149 countries and affect more than 1.4 billion people, costing developing economies billions of dollars every year." (WHO (NTD)). There is the opinion that these diseases are not only neglected because they have only an impact on the world's poorest people, but also because the focus was mainly on HIV/ AIDS, tuberculosis and malaria (Feasey *et al.*, 2010). Torgerson and Macpherson go so far to say that parasitic zoonoses have a similar human disease burden than one of the big three human infectious diseases, not even taking into account the burden in livestock industries (Torgerson and Macpherson, 2011). Furthermore the impact and management of neglected diseases need inter-sectoral collaboration which can be a challenge (Grace *et al.*, 2012; Molyneux *et al.*, 2011). However, funding NTD control shows high economic benefits (Feasey *et al.*, 2010; WHO *et al.*, 2006).

Table 1-1 Neglected tropical diseases (NTD) (WHO (NTD))

Causative pathogens	Disease
Protozoa	Chagas disease
	Human African trypanosomiasis (sleeping sickness)
	Leishmaniases
Bacteria	Buruli ulcer
	Leprosy
	Trachoma
	Yaws
Helminth	Cysticercosis/ taeniasis
	Drancunculiasis (Guinea-worm disease)
	Echinococcosis
	Foodborne trematodiases
	Lymphatic filariasis
	Onchocerciasis (river blindness)
	Schistosomiasis
	Soil-transmitted helminthiases
Virus	Dengue
	Chikungunya
	Rabies

In addition to the diseases mentioned in Table 1-1, there are other neglected conditions; chronic suppurative otitis media (CSOM), mycetoma, nodding syndrome (NS), podoconiosis, ectoparasites, snakebite and strongyloidiasis. The addition of chromoblastomycosis and other deep mycoses, scabies and other ectoparasites and snakebite envenoming to the NTD portfolio has been under discussion and they were added in 2017 (WHO (NTD)).

The greatest number of NTDs occur in Nigeria and the Democratic Republic of Congo (Hotez and Kamath, 2009). The majority of the NTD disease burden comes from helminth infections followed by schistosomiasis (Hotez and Kamath, 2009). Figure 1-5 gives an illustration of the geographical distribution of NTDs.



*Global Overlap of six of the common NTDs. Specifically guinea worm disease, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths, trachoma.

**Soil-transmitted helminth infections can be caused by three different worms, all treated the same way.

Figure 1-5 Global overlap of six of the common NTDs (CDC, 2011)

As already mentioned, one of the reasons why these diseases are neglected is because they occur in poor populations (Hotez and Kamath, 2009; WHO, 2011). Countermeasures may either be complicated and costly or the prevalence is estimated quite low. On the other hand, the under-reporting of cases might indeed keep the prevalence so low (WHO, 2011). A study was able to show that livestock keepers are more likely to seek health care at a later stage than people living far away from the health centre because of their animals (Kunda *et al.*, 2007; WHO, 2011).

The countries where neglected diseases are occurring often have low infrastructure and weak health systems, thus under-reporting is quite common (Fèvre *et al.*, 2008; Welburn *et al.*, 2015; WHO *et al.*, 2006). Fevre *et al.* provide an example for under-reporting for human African trypanosomiasis (HAT) caused by *Trypanosoma brucei rhodesiense*. He estimates a rate of 40% of under-reporting, also because HAT deaths are usually reported from hospitals, but not communities (Fèvre *et al.*, 2008). Therefore it has been difficult to get funding for control programmes against the individual diseases.

WHO produced a document in 2006 on a strategy for preventive chemotherapy in human helminthiasis (Crompton, 2006). This publication suggests an integrated approach for the control of several neglected diseases at once, focusing on drug administration. Baker *et al.* investigated the challenges and opportunities for the integration of control efforts (Baker *et al.*, 2010). Looking at lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis and trachoma, it becomes obvious that the epidemiological characteristics are very different. Another challenge is the overall goal of these programmes that can be either disease control or elimination (Baker *et al.*, 2010). Important points raised here too are the different requirements needed for the stakeholders involved. Policy makers are more interested in the economic considerations, whereas national programme managers need information for the organisation and campaign of programmes so that they adhere to the defined goals of WHO disease-control and elimination policy. Finally, the donor community is result-focused and needs estimates for duration and effectiveness of the programmes (Baker *et al.*, 2010).

In the context of neglected diseases, the 10/90 gap should be mentioned; 10% of global health research is used for diseases that account for 90% of the global burden (Stevens, 2004). Diseases prevalent in poorer countries are neglected also by the pharmaceutical industry (Stevens, 2004). One third of the global population and up to half the population in poor countries lack regular access to essential drugs, and if they do, the quality may be doubtful (Stevens, 2004).

In addition to NTDs, there are also the so-called neglected zoonotic diseases (NZDs). WHO defines zoonotic diseases "as diseases and infections that are naturally transmitted between vertebrate animals and humans. A zoonotic agent may be a bacterium, a virus, a fungus or other communicable disease agent." (WHO (NZD)). The original WHO list of NZDs comprised seven infectious diseases that are caused by bacteria, parasites and viruses. Some of these diseases overlap with the ones incorporated in the definition of NTDs, as seen in Table 1-2. Other diseases such as leishmaniasis, leptospirosis and food-borne trematodiases have been added to the list of NZDs, with a shorter list being currently targeted by WHO within the umbrella of its NTD control programme.

Table 1-2 Neglected zoonotic diseases (NZD) (WHO (NZD))

Causative pathogens	Disease
Bacteria	Anthrax
	Bovine tuberculosis
	Brucellosis
Parasites	Cysticercosis and neurocysticercosis (NCC)
	Cystic echinococcosis or hydatid disease
	Zoonotic sleeping sickness or human African trypanosomiasis (HAT)
Virus	Rabies

Zoonoses and emerging diseases from animals sum up to 26% of DALYs lost to infectious diseases and 10% of the total DALYs lost in poor countries, whereas in high income countries they are much less significant (Grace *et al.*, 2012). When deciding on control of priority diseases, Canning and Fevre *et al.* argue that a cost-benefit approach should be applied rather than looking at the burden of disease (Canning, 2006; Fèvre *et al.*, 2008). As an example, the cost of US$150 per DALY averted is attractive and of US$25 per DALY averted highly attractive (Fèvre *et al.*, 2008). In this sense, neglected tropical diseases should be targeted first (Canning, 2006; Fèvre *et al.*, 2008).

Another point to raise is the need for short and long-term activities (Welburn *et al.*, 2015). It is essential to continue control efforts even with decreasing incidence (Welburn *et al.*, 2015; WHO, 2011). In 2015, the expanded special project for elimination of neglected tropical diseases (ESPEN) has been created by WHO and partners to support affected countries through capacity building and financial support (Hopkins, 2016).

According to Faburay, significant decreases in malaria and HAT prevalence have been achieved during the past five decades but AAT and tick-borne infections are still high and on the rise. He recommends using a One Health approach to combat these diseases by capacity building of medical and veterinary personnel and more investment in research and development especially of vaccines (Faburay, 2015).

1.5 The role of livestock and animal health

Livestock have a much more important role for the livelihoods of people than often perceived. They contribute to the livelihoods of at least 70% of the world's poor (Ashley *et al.*, 1999; WHO *et al.*, 2006). The situation in Africa is more and more precarious since the growth in livestock production does not match the annual human population growth of 2.5% (Cardoso, 2012).

Livestock are essential to five main groups of people: owners, hired caretakers, vendors, consumers and those who work in related industries such as tourism and crafts (Minjauw and McLeod, 2003). Livestock are in some regions of the world the only possible way of getting food and generating cash in case of emergencies. The economic role of animals might be thought to be higher in rural areas, but that may be an underestimation, since urban areas very often depend on the agricultural products from rural areas. And these products either directly stem from animals such as meat and milk products or indirectly through production, by draught power or manure. Minjauw and McLeod divide the impact of livestock on livelihoods, negative and positive, into five categories; natural, social, human, physical and financial (Minjauw and McLeod, 2003; Randolph *et al.*, 2007). The natural assets involve soil enrichment but also erosion. Animals can be considered as financial assets because they are often considered the bank account of the owner through selling the animals or livestock products (Randolph *et al.*, 2007). Human assets are for instance the nutritional contribution but on the other hand the risk of zoonotic diseases. Physical assets are mainly realised through draught power and social assets are related to social status depending on the species, sex and number of animals owned (Minjauw and McLeod, 2003). In addition to that livestock are used in sociocultural transactions such as dowries or patronage.

Additionally, there is also the emotional factor of keeping animals which is barely ever mentioned. Being a livestock farmer is not just a job, it is a lifestyle and one has to have a certain passion and interest for it to be successful.

1.5.1 Livestock and animal health economics

At the micro-economic level, livestock economics studies the economic role of livestock in the household or farm economy, examining the costs of production, the values of output and how these are influenced by various factors linked to livestock productivity, notably animal health. To assess the economic burden of a disease in animals, we need to have information on how the disease affects their productivity and mortality (ideally comparing a situation with and without the disease). The impact of disease is also a function of what livestock keepers and sometimes the State are spending on various animal health measures. There is thus an important trade-off between livestock productivity and hence output and expenditure on animal health (Howe, 1985). Where new disease control interventions are being considered, their costs would have to be evaluated not only in relation to the losses due to the disease that can be avoided due to the measure, but also to the current costs related to indirect control such as vector control (for example trapping of insects and insecticidal treatment) and treatment (Minjauw and McLeod, 2003).

1.5.1.1 Gross margin analysis

A gross margin analysis evaluates and compares the profitability of the different component enterprises, such as livestock, various crops, etc. on a farm. It is calculated as output (which for livestock includes changes in the value and number of the animals held) minus the variable costs attributable to each enterprise. The period it is calculated for is commonly a year. In smallholder farming systems, however, it has limited applicability. It is mainly used by farmers who have quantitative enterprise data and who want to maximise their profits by allocating resources between enterprises (Rushton, 2009).

1.5.1.2 Partial budget analysis

When carrying out a partial budget analysis we assess if a change or an intervention would be beneficial for an enterprise. For this we calculate with the marginal costs and marginal benefits split up in the revenue foregone and the new costs against the costs saved and the new revenue. The revenue foregone is the income sacrificed by making a change and relates to the opportunity costs of an intervention. The new costs are the investment that has to be done in order to implement the change. On the other side, the costs saved are those that would have been paid if the project was not implemented. The new revenue is the extra income generated by the change in the enterprise (Rushton, 2009).

Beneficial change = costs saved + new revenue > new costs + revenue foregone

The strength of a partial budget analysis is that it is a useful technique for examining change. It is also useful when introducing time into analysis to assess change. A weakness is that it does not show the profit or loss of an enterprise as a whole, but the net increase or decrease in net income resulting from a proposed change. It is also not capable of examining seasonal resource flows and it is unsuitable for enterprises that have so-called ‘lumpy’ inputs and outputs which are irregular or fragmented or not easily subdivided (Rushton, 2009).

1.5.1.3 Herd Models

There are different types of models to simulate the effects of disease and disease control. Analytical herd models use observed data to investigate how closely they fit with a hypothesized relationship (Rushton, 2009). Optimising models investigate different strategies and input combinations to simulate how a production process could be improved. Simulation models are often used for benefit-cost analyses. They are divided into steady state and dynamic models. Dynamic models can be either stochastic or deterministic.

1.5.1.4 Value chains

Value chains describe the production and transport of a product from the beginning to the end, and the value added at each stage of the process. Several groups of people are involved in this activity to supply a specific product. Value chains are driven by consumer demand, because it is their money that pays for the activities done during the production process. Value chains follow national and local laws and cultural customs and are managed by the people who set the rules for the necessary processes (Rushton, 2009).

1.5.1.5 Benefit-cost analysis

Benefit-cost analysis compares the benefits and costs to the society as a whole of a project over time. The benefits and costs expected or earned in the future are converted to their ‘present value’ using a process known as ‘discounting’. The discount rate weighs future as against present sums, by effectively deducting compound interest from future sums at the discount rate. The discount rate is defined as the opportunity cost of capital, and in practical terms it can thus be seen as representing the minimum acceptable return earned in an alternative investment.

Traditionally, discount rates in livestock projects were usually between 8% and 12%, however in recent years the very low real interest rates (that is the market rate excluding the inflation effect) have led to lower discount rates being accepted. In the field of human health, the discount rate used for DALYs is usually 3%.

Once discounted, the present value of costs (PVC) is compared with the present value of benefits (PVB). There are then three measures that can be calculated which help to make a decision; the net present value (NPV), the benefit-cost ratio and the internal rate of return (IRR).

A project is acceptable when the NPV is positive, meaning that the PVB exceeds the PVC. It gives a good idea of the total profit of a project, but is not very useful in ranking projects.

The benefit-cost ratio is the PVB divided by the PVC, therefore an acceptable project should have a benefit-cost ratio greater than 1. It is a very good measure for ranking projects of different sizes.

The IRR is the discount rate for which PVB=PVC. It helps to compare projects, but certain conditions have to apply for it to be a valid figure (Putt *et al.*, 1988).

1.6 Environmental health and biodiversity

A pioneer in ecohealth or even One Health was Aldo Leopold. He used the word land health to talk about the landscape, plants and animals (Leopold, 1989).

An ecosystem is defined as a "dynamic complex of plant, animal and microorganism communities and the nonliving environment, interacting as functional unit. Humans are an integral part of ecosystems." (Leemans and de Groot, 2003). The ecosystem consists of multiple components that are interwoven and exist in an interdependent and balanced system. There are biological, alive components such as flora and fauna and there are chemical and physical influences to make life in an ecosystem possible. Those are climate, humidity, soil fertility amongst others. Any changes in this system have an impact on anything else in this system, as we can observe with the effects of El Niño and global warming on coral reefs for example (Glynn, 1993; Stone *et al.*, 1999). Ill-health of flora or fauna is also a sign for an imbalance with the exception of symbiotic parasite infections for instance (Hudson *et al.*, 2006; Marcogliese, 2005).

The convention of biological diversity defines biodiversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems." (CBD, 1993). Biodiversity is therefore a key indicator of a healthy ecosystem (Altieri, 1999; Keesing *et al.*, 2010; Keesing *et al.*, 2006).

Now, does a fragmented landscape with reduced biodiversity forcibly cause a disease threat to humans? An imbalanced ecosystem with less biodiversity threatens the health of all live components since it is reduced in resilience and immunological capacity (Acevedo-Whitehouse and Duffus, 2009; Altieri, 1999; Bernstein, 2014; Sandifer *et al.*, 2015). New emerging and re-emerging infections are suggesting that theory (Jones *et al.*, 2013; Keesing *et al.*, 2010; Keesing *et al.*, 2006). Many factors are made responsible for this effect; intensified agriculture (Jones *et al.*, 2013) and increased livestock-wildlife interaction (Bengis *et al.*, 2002). However, there are contrary opinions (Hough, 2014; Salkeld *et al.*, 2013). Hough argues that sometimes ecosystem dis-services are more beneficial to human health (e.g. improvement of socio-economic status) than biodiversity as such (Hough, 2014). Evolution changes the impact diseases can have on populations as was seen in medieval times with leprosy (Schuenemann *et al.*, 2013). Similar effects have been shown for trypanosomiasis since research found that an increase in human population leads to a decline in tsetse density (Reid *et al.*, 2000). The interactions within an ecosystem are so complex that it is easy to overlook relevant contributing factors and draw conclusions too fast and easily (Bernstein, 2014; Hudson *et al.*, 2006; Ridder, 2008).

Ferguson *et al.* highlight the fact that front-line vector control measures like insecticide-treated nets and residual sprays are not sufficient to combat malaria

(Ferguson *et al.*, 2010). Malaria eradication needs more knowledge on vector ecology and adapted interventions (Ferguson *et al.*, 2010; Tanga and Ngundu, 2010). Other vector control measures such as acaricides against ticks can even have a negative impact on the ecosystem, for example by creating resistances or killing beneficial insect species and aquatic life (Kunz and Kemp, 1994; Minjauw and McLeod, 2003).

1.6.1 Ecosystem services

The benefits people retrieve from ecosystems are called ecosystem services (Leemans and de Groot, 2003). The services can be divided into provisioning, regulating, cultural and supporting services (Coutts and Hahn, 2015; Leemans and de Groot, 2003). Provisioning services are water and food quantity and quality and medicine. Regulating services are air quality, infectious disease modulation and climate regulation. Cultural services are physical activity, mental health and social capital (Coutts and Hahn, 2015).

Human wellbeing depends on an intact ecosystem through the services it provides (Sandifer *et al.*, 2015; WHO, 2015). This has been expressed by an indicator in the USA (Smith *et al.*, 2013). But also human interaction with nature is seen as beneficial (Keniger *et al.*, 2013).

Climate variation and change has a huge impact on health and poverty of people, be it through natural disasters or warmer temperatures with better conditions for disease vectors. Weather and climate shape mosquito geographic distribution, population abundance, lifespan and transmission potential (Ali *et al.*, 2017; WHO, 2015). Also public sanitation and water management and human movement have a high impact on vector occurrence and impact from infectious diseases (Ali *et al.*, 2017; WHO, 2015)

The sustainability of an ecosystem is essential for its functioning (Leemans and de Groot, 2003). Some species contribute uniquely to the ecological balance in an ecosystem and therefore their loss is of greater concern (Walker, 1992). The extinction of animal species is often not intentional, but due to human activity and giving preference to other resources such as food, jobs, energy, money and development. The ecological, economic and cultural impact of an animal in an ecosystem is not obvious because of the complexity of ecological dynamics and changing cultural norms (Tewksbury and Rogers, 2014).

1.6.2 Role of wildlife in the disease cycle

The majority of emerging infectious disease events are zoonoses (60.3%) and 71.8% of them originate in wildlife (Jones *et al.*, 2008).

Infectious diseases at the wildlife-livestock interface have received an increasing amount of attention over the last decade (Wiethoelter *et al.*, 2015). For example, trypanosomes in wild animals have a high virulence. There is a clear indication that wild animals are a reservoir of these strains in eastern province in Zambia (Masumu *et al.*, 2012; Van den Bossche *et al.*, 2011). Therefore there are more severe infections the closer one gets to the national park (Masumu *et al.*, 2012; Van den Bossche, 2001). Overall, 14% of wild animals are infected with trypanosomes in the Luangwa Valley (Anderson *et al.*, 2011; Masumu *et al.*, 2012). By replacing wild animals as hosts for tsetse flies, other livestock like goats or pigs can play a role as reservoir hosts, because they exist in high numbers and are usually not treated (Masumu *et al.*, 2012; Simukoko *et al.*, 2007a). Therefore pigs and small ruminants will be the major factor for the circulation of trypanosomes between areas (Masumu *et al.*, 2012).

Buffaloes play possibly an important role in disease transmission between wildlife and livestock in Africa (Eygelaar *et al.*, 2015; Munang'andu *et al.*, 2009; Munang'andu *et al.*, 2006). This relates to tick-borne diseases, brucellosis, bovine tuberculosis, foot and mouth diseases and others (Munang'andu *et al.*, 2006). This fact makes it also impossible to keep African buffaloes on game ranches which would relieve the hunting demands for national parks (Munang'andu *et al.*, 2006).

However, with a high amount of positive but subclinical cases, cattle themselves might contribute to the spread of especially East Coast fever (*Th. parva*) (Yusufmia *et al.*, 2010). Some might develop a trypanotolerance and therefore trypanosomes keep circulating. There is a threat of high virulent strains from wildlife to pass through these species in the escarpment area and reach the plateau later (Masumu *et al.*, 2012; Van den Bossche *et al.*, 2011). Rhinoceroses (*Ceratotherium simum*) have also been found positive for theilerioses and may thus play a role as a reservoir (Govender *et al.*, 2011b; Nijhof *et al.*, 2003).

Figure 1-6 A cattle kraal and a goat house on the left are meant to protect livestock from wild animal attacks

In addition, various monkey species play a role as a reservoir for Zika virus (Ali *et al.*, 2017). Cristobal-Azkarate *et al.* found a high amount of antibiotic resistance in faecal samples from wildlife in Mexico. They also showed that terrestrial species were more affected than arboreal ones, and proximity to human dwellings increased antibiotic resistance (Cristobal-Azkarate *et al.*, 2014).

Wildlife impact on diseases does not only play a role in the countries where they live. Because of the high amount of illegal trade with bushmeat, which is a delicacy in expat communities all over the world, the diseases get exported to other areas (ProMED-mail, 2014d).

1.6.3 Land use change

The effect of land use change on infectious disease occurrence is still under investigation. Common anthropogenic drivers of disease such as encroachment and destruction of wildlife habitats and the spread of pathogens are a threat to biodiversity and human health (Cunningham *et al.*, 2017).

The unlimited use of natural resources and global climate change has contributed to the emergence of more zoonotic diseases such as Chagas, Hantavirus, rabies, fish tapeworms and marine vibriosis in Chile (Cabello and Cabello, 2008).

It has also been shown that deforestation is associated with elevated Zika virus risk (Ali *et al.*, 2017; WHO, 2015). Similarly, inadequate or damaged infrastructure as after an earthquake increases abundance of mosquitoes and hence the number of Zika virus cases (Ali *et al.*, 2017; WHO, 2015).

In the case of human intrusion into wildlife reservoirs the risk of infection with a zoonotic disease increases too. One example for this is the increase in Hendravirus cases in humans and domestic animals through human intrusion in fruit bat habitats in Australia (ProMED-mail, 2017a).

1.7 Holistic meaning of health - One Health

The term One Health has been used quite recently, but the concept behind has existed for over 50 years. The following sections explain the interpretation and application of One Health in more detail.

1.7.1 Historical development

As early as 1964, Calvin Schwabe, the initiator of the approach, defined the goal of One Medicine "to capture the interrelatedness between animal and human health, and the medical realities of preventing and controlling zoonotic diseases" (Schwabe, 1964; World Bank, 2010). Over time, the understanding of the One Health approach has widened and stands for a holistic and interdisciplinary approach that describes the complexities between people, animals, the environment and their health.

The concept needs cooperation of multiple disciplines and for this purpose FAO, OIE and WHO have signed a tripartite concept note (FAO *et al.*, 2010). FAO defines One Health as:

> "a collaborative, international, cross-sectoral, multidisciplinary mechanism to address threats and reduce risks of detrimental infectious diseases at the animal-human-ecosystem interface." (FAO, 2012).

Its application needs political will and binding policies in every country as well as internationally. The International Health Regulations (2005) have taken up the concept for the implementation and fulfilment of their core capacities (WHO, 2016). Today, One Health is represented by the collaboration of OIE, FAO, WHO, UNICEF, World Bank and UNSIC (Okello et al., 2011). In 2004, they published a document 'Contributing to One World, One Health: a strategic Framework for Reducing Risks of Infectious Diseases at the Animal-Human-Ecosystem Interface' in Manhattan that provides 12 rules for the prevention of epidemic and epizootic diseases and support the integrity of the ecosystem for the protection of animals, humans and biodiversity (FAO *et al.*, 2008).

1.7.2 The implementation of a One Health approach

Disciplines that contribute to the concept are amongst others environmental studies, conservation, anthropology, engineering, epidemiology, hydrology, microbiology, nutritionists, medical doctors, veterinarians, sociologists, public health scientists, meteorologists, migration experts, agriculturalists, entomologists, trade & transport, education, WASH, tourism, energy and finances (Galaz et al., 2015; Mazet et al., 2009).

The integration of data resources from different disciplines such as laboratory analyses, meteorological data and local reports from non-governmental organisations (NGOs) etc. can facilitate collaboration and team formation and for instance increase the sensitivity of the surveillance system (Molyneux et al., 2011; Scotch et al., 2009; World Bank, 2010). This type of data sharing can be done using E-Health tools (Scotch et al., 2009; Treerutkuarkul, 2016).

Most often the One Health approach is focused on zoonotic and neglected diseases. More than two thirds of zoonotic diseases are of wildlife origin (Mazet et al., 2009) and zoonotic outbreaks during the last 10 years have raised costs of 20 billion USD. Including the loss of trade, tourism and tax revenues, the sum amounts to 200 billion USD (Okello et al., 2011). Still, zoonoses are not considered a priority globally and in many countries their diagnostic capacity is not established. This leads to disease underreporting due to inability (Halliday et al., 2012; World Bank, 2010; Zinsstag and Tanner, 2008). Disease underreporting due to unwillingness is a problem when there are consequences on trade, reputation or lack of compensation (Halliday et al., 2012; World Bank, 2010). Globalisation has also an influence on disease dynamics and zoonoses are gaining importance in this context (Okello et al., 2011).

In addition, worldwide population growth increases the demand for livestock products and food security becomes a problem. Livestock production needs to grow by 50% until 2020 to satisfy the demand (World Bank, 2012). The increased production leads to a change in land use, agriculture and destruction of ecosystems (Mazet et al., 2009). The dependence of many countries on their agricultural sector together with the reduced productivity of fragmented landscapes contributes to poaching and further threat of ecosystems due to economic interests (Mbugi et al., 2012; Okello et al., 2011). At the same time, the destruction and change in ecosystems facilitates disease spread (Mazet et al., 2009; Okello et al., 2011).

Climate change also plays an important role in this vicious circle (Mbugi et al., 2012; World Bank, 2010).

Another problem is the lack of knowledge in the communities. People are not fully aware that animals transmit diseases and that people can get infected by sharing water resources or drinking raw milk (Mazet et al., 2009; Okello et al., 2011).

Furthermore the mindset of many stakeholders in animal and more often in human health is not adapted to this emerging situation. There is a lack of expertise in zoonotic diseases and a reluctance to work together, even more with non-natural scientists (Galaz et al., 2015; Mbugi et al., 2012; Molyneux et al., 2011; Okello et al., 2011). The value of social and socio-economic determinants of health as risk factors is even less appreciated (Galaz et al., 2015; Mbugi et al., 2012). The communities are not enough involved in response mechanisms and interventions (Mbugi et al., 2012). A suggested solution is the common education of stakeholders in One Health (Galaz et al., 2015; Mbugi et al., 2012).

The advantages of adopting the One Health approach can be manifold. It can have a positive impact on the wider system, not just one disease in one species (Zinsstag et al., 2007a; Zinsstag et al., 2007b). Also it provides the opportunity to save money by sharing resources, transport, reagents, joint trainings etc. (Mbugi et al., 2012; World Bank, 2012). Usually an epidemic would start in the animal population. The earlier an outbreak can be detected the more money can be saved to control it, as can be seen in Figure 1-7. Animals can be used as sentinels and the health system can react before the disease affects humans (Mbugi et al., 2012; World Bank, 2012 ; Zinsstag et al., 2007a; Zinsstag et al., 2007b).

Figure 1-7 Relationship between detection of a zoonotic disease and costs related to time of detection taken from (World Bank, 2012)

The use of vaccinations in animals for the prevention of a zoonotic disease in humans has been widely proven but is still not applied regularly (Monath, 2013). Since the different sectors are often still not working together, it is considered a preventative method to vaccinate humans but vaccinating animals is not even considered sometimes (Monath, 2013). Additionally, the vaccine quality and effectiveness varies between the species, vaccine and disease (Monath, 2013). Some infectious agents can also be transmitted through the environment and thus vaccinating animals does not protect humans (Monath, 2013).

Traditionally the focus is always on a pharmaceutical solution whereas a social or cultural intervention could be more effective and sustainable (Galaz *et al.*, 2015). An idea is also the integration of traditional medicine into the health system to enhance access to local people (in Tanzania 60% of the population use traditional medicine regularly) (Galaz *et al.*, 2015; Mbugi *et al.*, 2012).

In most countries, the problem is the funding of One Health activities by different ministries. This inhibits a coordinated and joint work as a team (Mbugi *et al.*, 2012; Okello *et al.*, 2011). The One Health concept is most often used for controlling diseases and not prevention, but it is proven that animal keeping has an impact on for instance malaria prevalence, a non-zoonotic disease (Franco *et al.*, 2014; Jaleta *et al.*, 2016; Mayagaya *et al.*, 2015; Okello *et al.*, 2011). Therefore, the concept should be used in a wider context (Galaz *et al.*, 2015; Okello *et al.*, 2011). Kamani *et al.* are of the opinion that Africa has highest chances to adopt the One Health approach since their systems are not yet fully institutionalised (Kamani *et al.*, 2015). Pearce is providing examples for a One Health application with non-communicable diseases (Pearce and Douwes, 2013).

According to Aguirre *et al.* the environmental part of One Health is the least developed one and they suggest to work closer together for example with the Society of Environmental Toxicology and Chemistry (SETAC) (Aguirre *et al.*, 2016).

Metagenomics help to understand the relationships between microorganisms and virulence genes in animals, humans and the environment. So even the inclusion of

One Health into molecular concepts enhances the understanding and fight against the burden of infectious diseases (Atlas *et al.*, 2010).

1.8 Diseases of importance in Zambia

In general, non-communicable diseases are playing an increasing role in Zambia as in most countries globally (Hall *et al.*, 2011; WHO, 2014). The most common infectious diseases in Zambia in animals and humans are mentioned in the respective chapters.

Relating to dog rabies in Zambia, it needs to be mentioned that every dog owner has to buy a licence for each dog from the Ministry or the local government. Officially, there is a maximum number of dogs anyone can keep. However, the law is not enforced therefore the actual number of dogs in the country is unknown.

1.9 Aims of the thesis

The main hypothesis is that wellbeing of people is influenced by poverty and health and that these also influence each other.

The text boxes in this thesis provide information that was mainly gained through observation or personal communication. Their intention is to enable a better understanding of the unique culture in the study area.

1.9.1 Research questions

What is the prevalence of the diseases of interest in livestock and people?

Has the disease prevalence in livestock changed since the study by Mubanga in 2006 (Mubanga, 2008) and is there an hypothesis that would explain this change?

Can we come up with a composite indicator of socio-economic welfare/ well-being, and relate this to disease status?

1.9.2 Work conducted

A round of informal key informant interviews was done by Dr Noreen Machila and the researcher in Lusaka in June 2012 to assess the situation and knowledge about trypanosomiasis in Zambia at policy level.

Then a census conducted in September and November 2012 builds the basis of this work. This census was done with the help of local people working as enumerators and several colleagues involved in the DDDAC project. During the census, information collected was on the location of the household, amount of people including age groups, the type of animals owned and complimentary information regarding their income and open comments/ questions to make.

The census was followed by participatory work conducted in April 2013. Focus group discussions were held by Dr Noreen Machila and attended by the researcher. The discussion was translated, where necessary, by local colleagues. Key informant interviews with representatives from the area were conducted by the researcher, partly with the presence of Dr Noreen Machila and Dr Neil Anderson. The results from this work was taken as a basis for the questionnaires, therefore they are mentioned here. The questionnaires were designed by the researcher, reviewed by Dr Noreen Machila and Dr Alex Shaw and piloted locally with support from Dr Martin Simuunza.

The researcher was in charge for the following field work of animal sampling, human sampling, conducting the household questionnaire and the field laboratory diagnosis. Local colleagues contributed greatly to the success of field work.

The molecular laboratory work was done by the researcher alone at the University of Edinburgh.

1.9.3 Work presented in this document

The general introduction in Chapter 1 commences this PhD research study with a short literature review defining some of the research terms used and describing the background of the work conducted.

The study area is described in Chapter 2 to provide an overview of the infrastructure and provisions in the area.

Following the participatory appraisals, a cross-sectional survey was conducted sampling humans and five animal species (goats, cattle, sheep, pigs and dogs) from 210 households. The households were selected randomly from a census conducted in 2012, with the objective of having two animal-keeping households for each one that had none. Thus roughly 140 households kept the above mentioned animal species and 70 households did not, with some discrepancies over time due to changes in animal holdings. The samples were tested for selected diseases and a health questionnaire was administered for every sample taken in both people and animals. The results for the animal samples can be found in Chapter 3 and for the human samples in Chapter 4.

Furthermore, each household was asked about wellbeing of the people living in the household. These questions were compiled from observations of prior visits to the study area, information from the literature, topics raised during the FGDs & KIIs and experiences with questionnaires in other countries. A descriptive summary of the answers is provided in chapter 5.

A general discussion where all relevant findings are brought together is provided in Chapter 6.

1.9.4 Software used

The software programmes used for this work are outlined below.

- Questionnaires during fieldwork: five Samsung tablets using droidSurvey v2.5.2 for questionnaire design
- Random household selection: Research Randomizer
- GIS software: QGIS v2.0.1
- Statistical software: Epi Info 3.5.3, Winpepi 11.39, Minitab 17, Csurvey 2.0, SPSS 21 & Amos
- Graphpad was used for simple statistical calculations of significance
- Spreadsheet software: Microsoft Office 2007
- Reference software: Endnote X2
- Calculation of design effect and clustering with Epi Info 3.5.3

1.9.5 Ethical consent

The ethical consent for this study was granted from ERES Converge in Lusaka, Zambia, under the reference number 2012-Oct-002. In addition, each household member signed a consent form to agree to the sampling of people, animals and the answering of the questionnaires.

In addition to that, the four Chiefs of the study area (Chief Kakumbi, Chief Jumbe, Chief Msoro and Chief Mkhanya) were visited and asked for agreement and support before the survey started.

1.9.6 Papers already published

Exploiting Human Resource Requirements to Infer Human Movement Patterns for use in Modelling Disease Transmission Systems: an Example from Eastern Province, Zambia. Alderton S., Noble J., Schaten K., Welburn S. & Atkinson P.; PLoS One. 2015 Sep 30;10(9):e0139505.

A Multi-Host Agent-Based Model for a Zoonotic, Vector-Borne Disease. A Case Study on Trypanosomiasis in Eastern Province, Zambia. Alderton S, Macleod ET,

Anderson NE, Schaten K., Kuleszo J, Simuunza M, Welburn SC, Atkinson PM. PLoS Negl Trop Dis. 2016 Dec 27;10(12).

Local disease-ecosystem-livelihood dynamics: reflections from comparative case studies in Africa. Leach M, Bett B, Said M, Bukachi S, Sang R, Anderson N, Machila N, Kuleszo J, Schaten K, Dzingirai V, Mangwanya L, Ntiamoa-Baidu Y, Lawson E, Amponsah-Mensah K, Moses LM, Wilkinson A, Grant DS, Koninga J. Philos Trans R Soc Lond B Biol Sci. 2017 Jul 19;372(1725).

Schaten, K.; Shaw, A.P.M.; Machila, N.; Simuunza, M.; Chirwa, E.; Anderson, N.E.; MacLeod, E.; Welburn, S.C. (2017). Economic and social science questionnaire dataset, Mambwe District, Zambia (2013). NERC Environmental Information Data Centre. https://doi.org/10.5285/b1647138-49f5-4777-a39d-e7359bf7b98d.

Schaten, K.; Machila, N.; Chirwa, C.; Shaw, A.P.M.; Mashili, J.; Hang'andu, F.; Simukonda, S.; Simuunza, M.; Anderson, N.E.; MacLeod, E.T.; Thrusfield, M.; Welburn, S.C. (2017). Domestic animal health data from Mambwe District, Zambia (2013). NERC Environmental Information Data Centre. https://doi.org/10.5285/f81ede76-a1d4-4367-aa8c-de087350457e.

Chapter 2: Infrastructure of the study area

This chapter provides an overview of the environmental, socio-economic and ethnographic features of the study area in Mambwe District; it is partially based on selected literature, but relies mainly on personal observation and communication with the local inhabitants.

2.1 Geography

Zambia is a landlocked country in Southern Africa. It is bordered by the Democratic Republic of Congo in the North, Tanzania in the North-East, Malawi in the East, Mozambique, Botswana, Namibia and Zimbabwe in the South and Angola to the West. It is divided into ten provinces; Luapula, Northern, Muchinga, Eastern, Lusaka, Copperbelt, North-Western, Central, Western and Southern province, which are again divided into 89 districts and further into constituencies, wards and standard enumeration areas (SEAs).

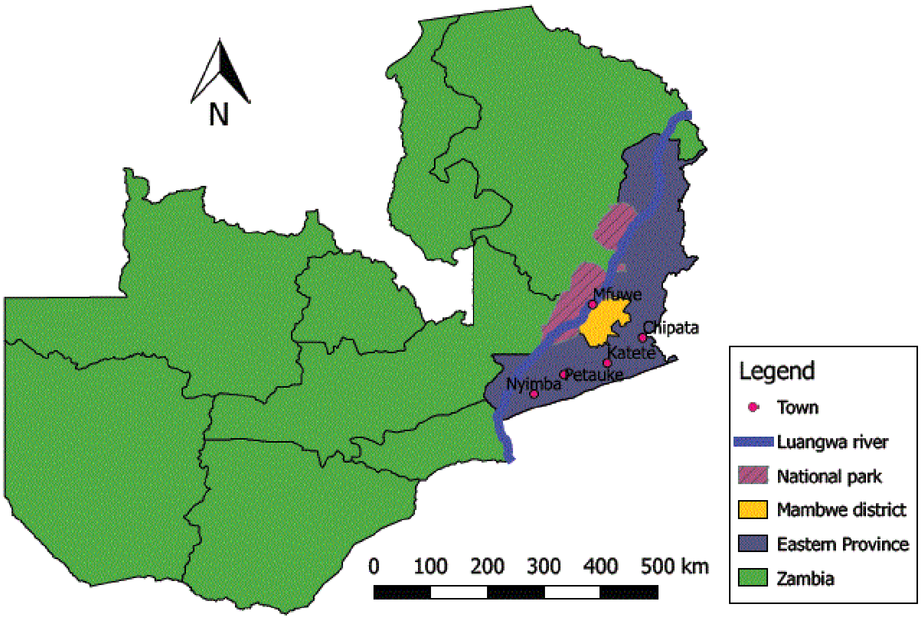


Figure 2-1 Map of Zambia, showing the location of the study area

The study was conducted in Mambwe District, see Figure 2-1, which is one of the nine districts in the Eastern Province. The other districts within Eastern Province are Katete, Nyimba, Chadiza, Chipata, Lundazi, Vubwi, Sinda and Petauke. To reach the Eastern Province from Zambia's capital Lusaka, one has to pass the only bridge crossing Luangwa River along the Great East Road (Figure 2-2). The Great East Road stretches as a straight line along the plateau from Lusaka to the Malawian border passing several towns including Nyimba, Petauke, Katete and Chipata, the capital of the Eastern Province. The traffic on the Great East Road has increased significantly over the past few years due to the volume of trade within Zambia, but also to trade links between Zambia and the rest of South-East Africa. This has led to a growing population with an increasing number of businesses along the road. There is also a big development in the tourism sector with the provision of accommodation and restaurants of different price categories where travellers can stop. The main attractions of the Eastern Province are its four national parks; the North Luangwa National Park (NLNP), the South Luangwa National Park (SLNP), Luambe National Park and Lukusuzi National Park.

Figure 2-2 The GreatEast road on the plateau linking the border of Malawi via Chipata, Katete, Nyimba to Lusaka

Mambwe District stretches along the Luangwa River in the Luangwa Valley. It is confined by two plateau areas, one to the North West called Muchinga escarpments and one to the south east. The source of the Luangwa River is in the Lilonda and Mafinga hills near the border with Tanzania and Malawi and from there it continues to become the fourth biggest river in Zambia till its final destination, the Zambezi River. Within the valley, it is a powerful river during the rainy season branching off several streams such as the Lupande River. Mambwe District shares a border to the west with one of the most famous and largest national parks in Zambia, the SLNP.

The SLNP covers some 9050 km^2 and rises from 600-1000 m in altitude (Nyirenda *et al.*, 2008). It is famous for a variety of wildlife species which will be mentioned later in this chapter. The only entrance to SLNP is at Mfuwe crossing the Luangwa River. Mfuwe is a growing town with its own international airport, thriving on the increase in tourism. There is also a newly built road connecting the Great East Road from Chipata to Mfuwe. Mfuwe Airport is the only international airport in the Eastern Province (ZAWA, 2011).

Figure 2-3 Most roads in the valley are in poor condition

Leaving Mfuwe, a road runs along a transect line to Katete, however, the roads in the area are often in poor condition (Figure 2-3). Within a distance of 10 km to each side of the road the households that participated in this study can be found (Figure 2-4). This transect line was chosen based on the study area Dr Joseph Mubanga used for his PhD in 2007 {Mubanga, 2008 #793}. It lies in the Lupande Game Management Area (GMA) which surrounds the SLNP and still harbours a lot of wildlife. A GMA is less restrictive than a national park and allows limited agricultural activities under certain conditions. The Lupande GMA is divided into the Upper Lupande, Lower Lupande and Msoro hunting blocks (ZAWA, 2011).

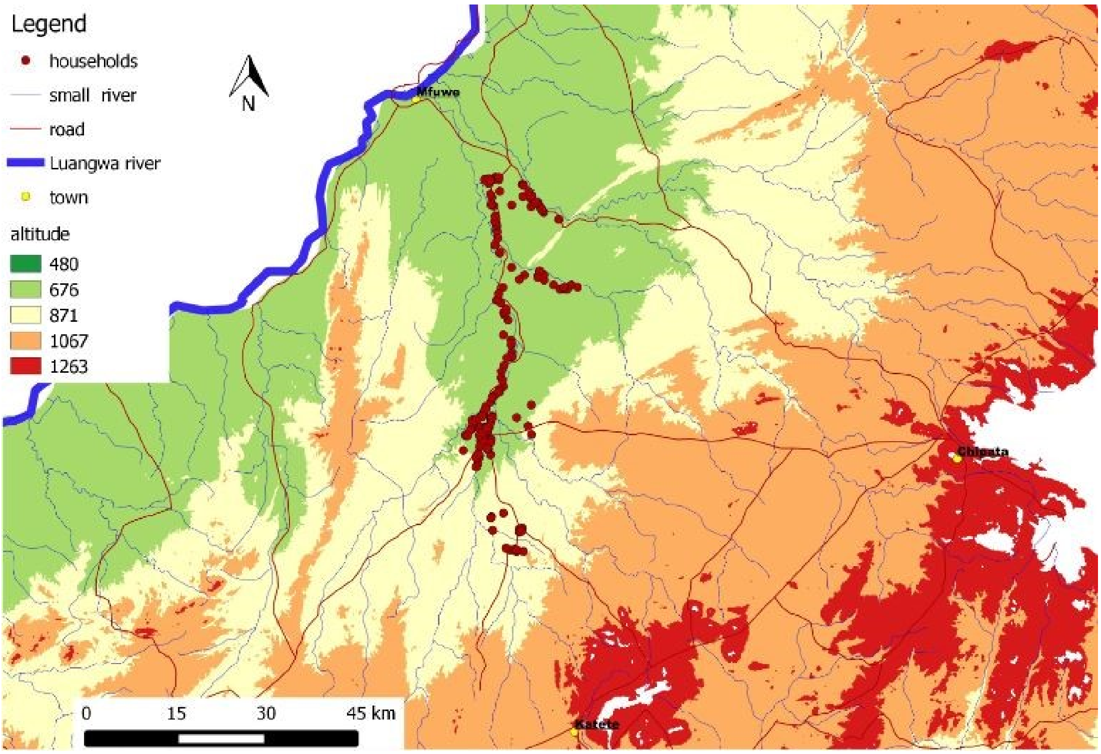


Figure 2-4 Map with households included in the survey

The infrastructure of the study area is poor and the roads are extremely bad, a challenge for any vehicular transport (ZAWA, 2011). Sometimes even using a bicycle poses a risk, but the inhabitants of this area are skilled cyclists and are able to use these roads even when transporting bulky luggage (Figure 2-5).

Figure 2-5 A bike is the most important mode of transport in the area (© J. Kuleszo)

The transect line crosses the Lupande river twice, which is impassable during the rainy season. During the dry season, the river dries out, but because the river bed is very sandy and loose, it is still a challenge to cross it by car or truck without getting stuck (Figure 2-6).

Figure 2-6 When the car is stuck in the dry riverbed, everybody has to help (© J.Kuleszo)

The only regular traffic travelling along this road are the trucks of the cotton companies. With their heavy load, they may even contribute to further destruction of the roads.

2.2 Climate

The study area has three seasons during the year. First is the rainy season, which is also the planting and harvesting season and starts from November till April. The following cold season is dry and cool at night and lasts from April to early August. From the end of August till November is the hot and dry season when the weather is hot and temperatures are increasing progressively.

The mean minimum and maximum temperatures in the study area range between 15°C in the cold season to 36°C in the hot and dry season. The average annual rainfall is between 400-800 mm but can go up to 1000 mm in exceptional situations (ZAWA, 2011).

During the rainy season, it is quite common for the rivers to overflow and the area becomes flooded. During a severe flood in 2012, inhabitants of the study area lost all their belongings and their harvests.

In these situations, the local people are supported by a wide network of international organisations (IO), non-governmental organisations (NGOs) and charities by providing them with survival essentials, such as tents, to lessen the burden of such calamities.

In some years drought poses a greater risk to the inhabitants, because they cannot harvest enough crops to nourish a family until the next harvesting season.

2.3 Demography

The inhabitants of the area were originally subsistence hunters, but now they try to sustain themselves by crop farming. The study area is not ideal for livestock keeping, because the burden of tsetse fly transmitted trypanosomiasis is too high (Carr, 1996; Dalal-Clayton and Child, 2003; Marks and Fuller, 2008; Marks, 1976). The following sections will give a brief overview of the population and its culture.

2.3.1 Traditional societal system

The Luangwa Valley has six chiefdoms (Figure 2-7), namely Jumbe, Msoro, Kakumbi, Mnkhanya, Malama and Nsefu (Dalal-Clayton and Child, 2003; ZAWA, 2011). The chiefs communicate with the communities through administrative helpers known as Indunas and they again have assistants, the headmen. Each village has a headman and an Induna is the hierarchical connection between a chief and the village headmen. A village in this context is a collection of households that can be, but does not have to be geographically close. Usually inhabitants of a village are all part of one family.

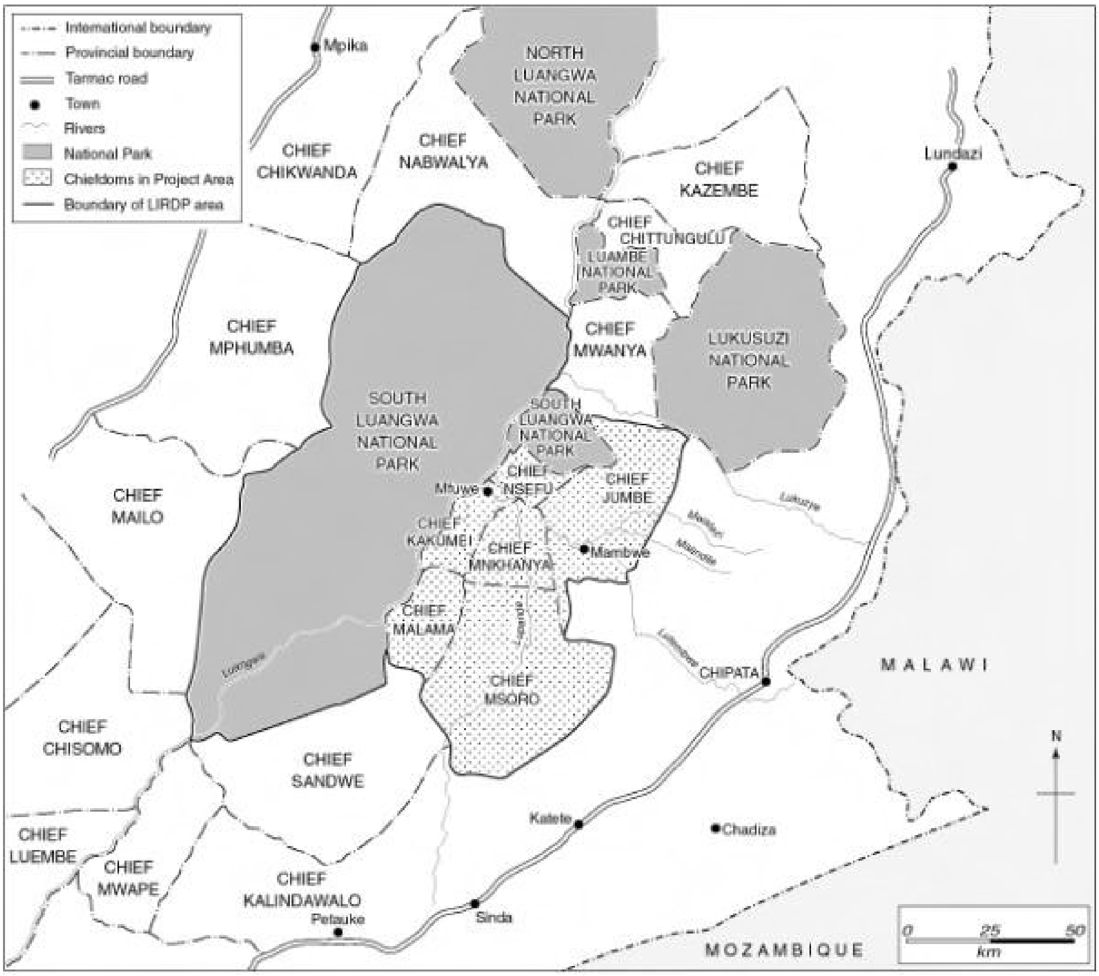


Figure 2-7 Geographical distribution of chiefdoms in the Luangwa valley (Dalal-Clayton and Child, 2003)

Nowadays, the role of the traditional system is to manage land distribution and to judge in civil cases involving various forms of conflict (ZAWA, 2011). It is supported by an area councillor and local Member of Parliament. However, the role of traditional rulers in natural resource management has been changing over time and resulted in less control nowadays (Pope, 2005).

2.3.2 Ethnicities

There are several different tribes in Mambwe District; the two main tribes are the Kunda and the Chewa. The Kunda are believed to be successors of the Bisa, a people originating from the valley (Marks and Fuller, 2008), but have closer affinities with the Nsenga (ZAWA, 2011). They have a big traditional ceremony called Malaila which is held annually around August and September. People travel from far and wide for several days to participate. This ceremony symbolises the importance of communal efforts in overcoming problems facing the community. It includes the performance of a fake lion hunt where as a culmination of the festival the ‘dead lion’ is presented to the Kunda chiefs (ZAWA, 2011).

The Chewa came originally from the Democratic Republic of Congo and Malawi. They have well-developed agricultural techniques. They are closely related to the Tumbuka, Bemba and Nsenga, and known for their masks and secret society culture (Nyau).

The other tribes of people found in the area are the Ngoni, Nsenga, Senga and Lenje. The Ngoni people have their origin from the Zulu in South Africa and migrated up north a long time ago. The Nsenga are distinct from the Senga people. They originated from Zambia and Malawi and are mainly found on the plateau area. They are craftsmen and crop farmers. In contrast, the Senga people are believed to come from the Democratic Republic of Congo and are mainly found in the Eastern province of Zambia.

Relatively few Lenje are living in the area, they are more found in Central province. This people is believed to have its origin in Cameroon. They are farmers.

Text box 2-1 Chief Tindi and the Chewas

The Chewa originate from Malawi. They are traditionally cattle farmers who usually keep large herds and therefore need a lot of space to have enough grazing for their animals. Some 10-20 years ago, a man persuaded many Chewa to move to the area around Tindi. He promised them more and better land, provided they would come with him and support him to be a new Chief in the area. To this day, the self-proclaimed Chief Tindi is not accepted by the other chiefs nor by most inhabitants of the area. People are even reluctant to talk about him and call him by this name. The introduction of this new ethnic culture exerted a remarkable influence in the valley from merely subsistence hunting to now subsistence farming and big business poaching.

2.3.3 National census data

Zambia previously consisted of nine provinces, however, in 2011 an additional province, Muchinga, was formed from Chinsali, Isoka, Mafinga, Mpika, and Nakonde districts of Northern and Chama district of Eastern Province. Each province is subdivided into districts, each district into constituencies, and each constituency into wards. In addition to these administrative units, during the 2000 population census, each ward was subdivided into convenient areas called census supervisory areas (CSAs), and in turn each CSA into standard enumeration areas (SEAs). In total Zambia has 89 districts, 150 constituencies, 1,289 wards, about 4,400 CSAs, and about 16,400 SEAs.

Zambia conducts a national census every 10 years. It is supported by international organisations such as the United Nations Statistics Division (UNSTATS). The questions asked and the data to analyse are immense which is why some of the analysis of the last census is still ongoing. Information about the data from the 1990 to 2010 census is given in Table 2-1. As can be seen, the national census data are not conclusive. It was attempted to find an explanation for the irregularity by looking at several source documents and by contacting the Central Statistical Office (CSO) directly. It would have been interesting to know where and how the irregularity occurred. However, my efforts to understand this problem were precluded as I was unable to receive a satisfactory answer from the CSO. It seems however, that the data between Nyimba District and Mambwe District were muddled at some point which is why the data for both districts are presented in the table. Interestingly, Pope also found this discrepancy in the Luangwa Safari Association tourism study and it is also unexplained (Pope, 2005). Other sources however mention a big immigration into the study area and its major influence (Mubanga, 2008).

The table uses *de jure* and *de facto* populations. The *de jure* population is defined as

> "all usual residents, whether or not they are present at the time of the enumeration.",

whereas the *de facto* population

> "consists of all persons who are physically present in the country or area at the reference date, whether or not they are usual residents." (UNSTATS, 2015).

Table 2-1 National census population data from 1990-2010 (Central Statistical Office Zambia, 2003; Central Statistical Office Zambia, 2011; Central Statistical Office Zambia, 2012a; Central Statistical Office Zambia, 2012b)

NATIONAL CENSUS OF ZAMBIA	2000 census summary report (Nov 2003)	Found on page	2010 census of population & housing preliminary report (Feb 2011)	Found on page	2010 census of population & housing population summary report (June 2012)	Found on page	2010 census of population & housing descriptive tables (Nov 2012)	Found on page
1990								
EP total								
EP HH								
Mambwe total	60 016	16						
Nyimba total	38 300	16						
2000								
EP total	1 306 173		1 306 173	16	1 231 283	19	1 231 283	22
EP HH	254 603							
Mambwe total	47 376	18, 31	47 476	16	47 376	19	47 376	22
	70 425	16						
Nyimba total	70 425	18, 32	70 299	16	70 425	19	70 425	22
	47 376	16						
2010								
EP total			1 707 731	16	1 592 661	114	1 592 661 dj (1 525 123 df)	2, 4
EP HH			341 443		305 198	114	305 198 dj	7
Mambwe total	68 918	52	71 074	13,16,20	68 918	19	68 918 dj (64 672 df)	2, 4
Nyimba total	85 025	52	85 684	13,16,20	85 025	19	85 025 dj (81 025 df)	2, 4

dj=de jure, df=de facto, EP= Eastern Province, HH= households

2.4 Flora and fauna in the valley

More than 30% of Zambia's land mass is protected for wildlife through either the 19 national parks or the game management areas. There are four national parks in the Luangwa valley; the NLNP, Luambe National Park, Lukusuzi National Park and the SLNP. The SLNP is the biggest of them and visited by most tourists in Zambia.

Figure 2-8 Entrance to the South Luangwa national park

In general, the number of trees, reeds, fish and wild animals is decreasing with concomitant impact on the peoples' livelihoods. Fish are mainly threatened through the use of incorrect fishing gear, and trees and reeds are overused for charcoal production and exploited for mats, baskets, etc. or they are destroyed through increased human settlement (ZAWA, 2011). Defining correct environmental land use practices are vital for securing the region's biodiversity (Pope, 2005).

2.4.1 Wildlife

The Luangwa Valley historically has been rich in wildlife. Despite the fact that many animal species have reduced numbers for reasons that will be explained later, with the exception of rhinoceros, four of the 'Big five', elephant, buffalo, lion and leopard, can all be found. Cheetahs are found only in NLNP.

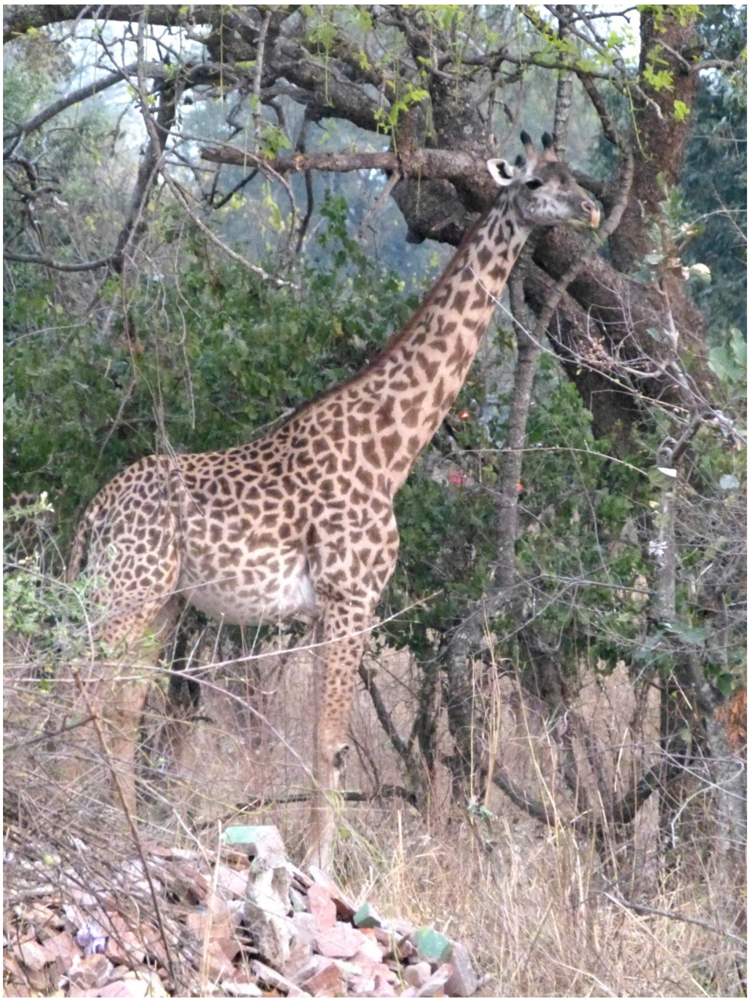


Figure 2-9 The Thornicroft giraffe is unique to the Luangwa valley (© J. Kuleszo)

Cookson's wildebeest and the Thornicroft giraffe (Figure 2-9) are local species in the valley (Nyirenda *et al.*, 2008). Other common species are hippopotamus, hartebeest, zebra, eland, sable, kudu, roan, waterbuck, impala, puku, reedbuck, warthog, baboon and monkeys. Additionally, the valley hosts over 700 species of birds (ZAWA, 2011).

2.4.1.1 *Aerial surveys and wildlife population trends in and around the South Luangwa National Park*

Several surveys were conducted irregularly in the Luangwa Valley with different area coverage (2014; Frederick, 2013). The last survey in 2012 comprised flying transects with spacing of 2.5-10 km depending on wildlife densities. The total area covered was approximately 30,418 km^2. Observers were employed for counting the different animals as well as imaging support and calibrated counting strips on each side of the aircraft. Unfortunately, these methods are not always very accurate, since they depend on the ability of the observer to spot animals, technological problems or just plants blocking the view. Also, animals showing up in high densities or rare, small and night-active animals are difficult to count reliably (Figure 2-10). Due to their similarity, impala and puku created some identification issues. Live elephants as well as elephant carcasses were counted.

Kudu, impala, puku, roan, zebra and warthog showed significant declines since the last survey in 2009. Even more severe is the drop in elephant population, especially in the Lupande and Lumimba region (-75%). SLNP shows also more elephant carcasses than NLNP. Different numbers of carcasses were found when walking and flying which suggested an underestimation of the overall number of carcasses (Frederick, 2013). Pope expresses a severe warning for the depletion of the elephant population. Nevertheless, in spite of this view there has been a proposal from ZAWA to reintroduce elephant hunting legally in Zambia (Pope, 2005).

Figure 2-10 A dried out riverbed is a good place to observe different species of wildlife (©J.Kuleszo)

Dalal-Clayton *et al* report on earlier figures, where the black rhino population was reduced from 8,000 in the 1970s to less than 100 in the 1980s and none nowadays. Similarly, the elephant population has reduced from 90,000 to 15,000 down to 2,500 in 1989. This sharp decline was due to a poaching of on average 9 elephants per day alone in the SLNP. In 2003, the population had increased again to an estimated 9,000 animals. A hunting ban in 2001 and 2002 institutionalised by President Chiluba himself might have well contributed to this development (Dalal-Clayton *et al.*, 2003).

Wasser *et al* have found a way to identify genetically where ivory originates from. Hopefully, their method will be increasingly useful to impede further elephant ivory poaching and trade (Wasser *et al.*, 2015).

2.4.2 Vegetation

The ecosystem plays a vital role in disease maintenance and transmission. It also contributes significantly to the wellbeing and poverty of its inhabitants (Bernstein, 2014; Horton and Lo, 2015; Hough, 2014; Keniger *et al.*, 2013; Ridder, 2008; Wiethoelter *et al.*, 2015).

The primordial vegetation in the valley is grass or shrub savannas (munga) with mopane (*Colophospermum mopane*) woodlands in the valley and miombo (mainly *Brachystegia spp.*) woodlands on the escarpments. The tree species most often found in munga are *Acacia*, *Combretum* and *Terminalia* spp. Miombo woodlands provide supply for timber, firewood and charcoal (Sekeli and Phiri, 2002). These woodlands are a preferred habitat of tsetse flies, the vector for trypanosomiasis (Van den Bossche and De Deken, 2002).

Unfortunately, the charcoal trade has led to a severe depletion of forest resources (Dalal-Clayton and Child, 2003; Gumbo *et al.*, 2013). Poverty, unemployment and lack of other income options are the main drivers for charcoal trade. Progressive urbanisation will even aggravate the situation as there are not many other sources for heat and cooking available (Gumbo *et al.*, 2013).

Figure 2-11 Mopane woodland (© J.Kuleszo)

2.5 School system

The Zambian school system starts with primary education which goes from Grade 1 to Grade 7 and terminates with an exam for the Certificate of Primary Education (CPE). This certificate decides where the pupil continues to Junior Secondary (Grades 8 and 9). At the end of Grade 9, there is another exam which decides for the continuation of Grade 10 to 12, Upper Secondary (Project-Luangwa). With only two secondary schools in Mfuwe and around 18,000 children in the area, most pupils only remain in school until Grade 7. Some will continue at the local basic school for grade 8 and 9. Those parents who can afford it will send their children to boarding schools to access secondary education (Project-Luangwa).

The school year runs from January to December and school lessons are usually given in shifts. Because there are too many children in one class, the school tries to lower the burden by teaching one set of pupils in the morning and another in the afternoon. In more remote areas, local untrained volunteers teach children in the so called community schools.

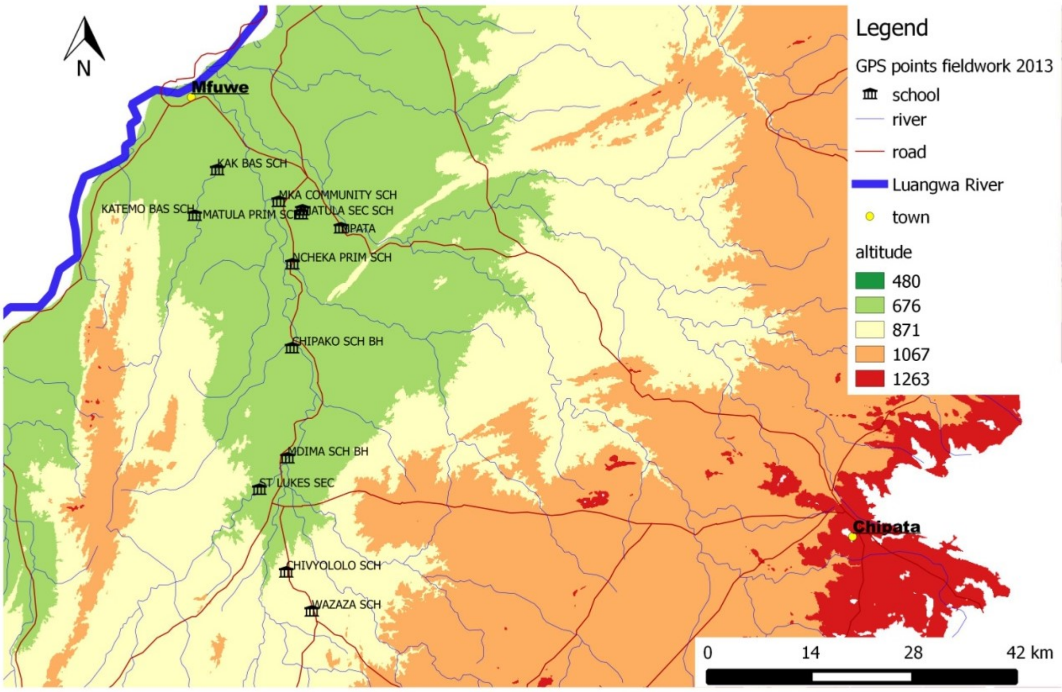


Figure 2-12 Schools in the area (GPS points taken during fieldwork therefore no claim to be complete)

Problems with getting to school, lack of funds for uniforms, exercise books and pencils cause low attendance rates. Very few of the people are also able to pay the school fees from grade 8 onwards (Project-Luangwa). Mambwe district has one of the lowest school attendance rates in the country of only 18% (national 26%), which results in general literacy and trained personnel rates of only 38% (national 55%) (Pope, 2005). Literacy is mostly at primary level.

2.6 Health in Zambia

It is very helpful to hire local people as personnel for the health system. They have the interest in improving the health situation of people in the area and at the same time enjoy the public confidence more than health workers from other parts of the country, belonging to other tribes and acting officially as government agents (Evans, 1996).

2.6.1 Health centres

The health services in Mambwe district are below average (Pope, 2005). The 12 health centres in the area are relatively well distributed, but households still have to cover large distances to access the nearest health centre, in the absence of public transport. The services provided are part of the primary health care. There are two larger hospitals for secondary health care which are in Msoro (St Lukes Hospital) and Mambwe (Kamoto Mission Hospital), as shown in Figure 2-13. For more serious medical conditions or to get a special diagnosis or treatment, people need to go to St Francis hospital in Katete or the district hospital in Chipata, which both provide tertiary health care.

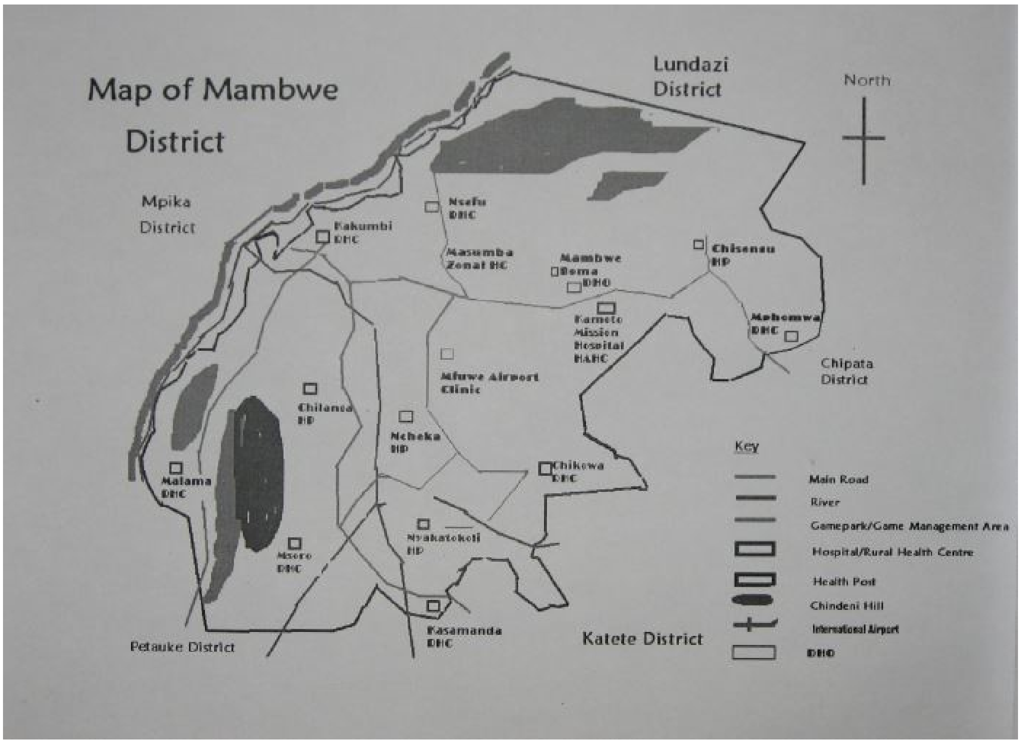


Figure 2-13 Health centres in the area

The main health services provided in the primary health centres are HIV/AIDS prevention, static and mobile anti-retroviral treatment (ART), child health, maternal health, environmental health, malaria, breastfeeding, nutrition and non-communicable diseases. Kamoto mission hospital has only one doctor and is lacking transport and equipment (ZAWA, 2011).

2.7 Agriculture in the Eastern Province

The main type of farming in the study area is subsistence farming, i.e. most animals raised and plants grown are for the peoples' own consumption. Sometimes animals, especially cattle, are seen as a long term investment and therefore very valuable. This is a well-known method in developing countries for people to 'have savings in their bank account' (Freeman, 2008). In the case of the Luangwa Valley, even dogs have their value, because they are often used for hunting. More information on the role of dogs will be provided in the animal health chapter.

Figure 2-14 Slash-and-burn agriculture (© J. Kuleszo)

A common practice in the Eastern province is to burn down the grass and bushes that have grown during the year before the rainy season. This will clear the fields for the next planting season. Furthermore, the soil will get nutrients from the ashes. However, nowadays many areas are burnt at least twice a year. Cattle farmers like this practice, because new grass will grow on the burnt areas and they will have more feed for their cattle. Scasta goes so far as to say that controlled fire reduces the risk of vector-borne diseases (Scasta, 2015). Other people appreciate the slash-and-burn agriculture, because it will facilitate the hunting of rodents that are considered a delicacy in the area (see Text box 2-2). However, ZAWA claims that fire can cause severe damages to the ecosystem too (ZAWA, 2011).

Evidence of public-private partnerships in agriculture and agricultural education is scarce in the study area. However, it would promote farmers' knowledge and methods and thus provide the possibility to enhance the socio-economic standard in the area (Evans, 1996).

2.7.1 Crop farming

Nearly every inhabitant of the study area grows their own vegetables or plants for private consumption. Some focus their production on certain plants to have an advantage in selling them, but then need to buy others. Maize will usually be stored by a household and consumed until the next harvest is due. A bad harvest in the rainy season might lead to the stored maize being used up more quickly, so that people will suffer from malnutrition in the dry season until the next harvest is collected (Freeman, 2008).

Figure 2-15 A former wood cleared for farming (© J.Kuleszo)

2.7.1.1 Crop and vegetable production for food

The main crops grown for food consumption are maize, sorghum, millet and sweet potatoes. Plants adding up to the diet are groundnuts, cow peas, beans, sunflowers, pumpkin leaves and rape leaves. Typical vegetables and fruits grown in the area are tomatoes, onions, cucumbers, bananas, papayas (*pawpaw*), mangoes, sugar cane and cabbages (ZAWA, 2011).

The Wildlife Conservation Society (WCS), whose work will be described later, realised how often incorrect land use practices were having a deleterious effect on the ecosystem and thus created the Community Markets for Conservation (COMACO). It is a model to get people involved in finding solutions to save natural resources across the whole area. They sell their products such as rice, honey and peanut butter nationally.

Figure 2-16 Pack of rice produced by COMACO

Fish, wild fruits and mushrooms are complimenting the diet (ZAWA, 2011).

2.7.1.2 Cotton production

Cotton is a typical cash crop in the area. It is grown only to generate money and does not have any other purpose (Freeman, 2008). Formerly, cotton was processed in Zambia, but nowadays the raw cotton is exported mainly to Asia for further refinement.

Cotton production in Zambia received much media attention during the past few decades. There were big price changes, leading to riots where farmers were even burning their harvest as a manifestation of their protest. The quality of Zambian cotton was previously similar to that of Egyptian cotton, but has declined substantially more recently (personal communication Dr Joseph Mubanga). It is not clear if the decrease in quality comes from the farmers' management methods or the leaching out of the soil, different type of cotton seeds, climate etc. Fertilizers are for many farmers a limiting factor, since their correct usage is very costly (Freeman, 2008).

The farmer needs to decide if he prefers to grow cotton or maize. For both plants, the farmer needs a considerable amount to either feed the whole family for the rest of the year and in good harvest times even sell some maize, or to harvest enough produce for the cotton company to pay a better price (Freeman, 2008).

Figure 2-17 A cotton field ready to harvest in April

Fire clearing is traditionally done before the planting season in November to free the fields of weed and all unwanted plants. It is supposed to help fertilize the soil as well.

The system of cotton production in Zambia is the same for all companies. The farmer is provided with seeds, fertilizer, insecticide and all other equipment needed for the amount of land under cultivation before the planting season. They start therefore 'in debt' to the company. When they have harvested the cotton, the company will collect it, the cost of the materials supplied as an advance will be subtracted from the value of the crop and the remaining revenue is paid in cash. This is usually a great time for the family and it is not uncommon that part of the money will be used for celebration.

2.7.2 Livestock production

Livestock kept in the area are cattle, goats, sheep and pigs. Nearly everybody keeps poultry either chicken, duck or guinea fowl. Many households also keep a dog for protection or hunting. The traditional cattle in Zambia are mainly Sanga and Zebu (Perry *et al.*, 1984).

Figure 2-18 Guinea fowls are the most valuable poultry kept in the area (© J.Kuleszo)

2.7.2.1 Grazing and water

Nearly all animals are free roaming, except for a few big cattle herds that are brought to grazing grounds. Therefore resources are 'free' to everyone. There is no control over the feed or breeding of any species. Overnight most farmers keep their animals in so-called *kraals*, because that way they are better protected from attacks from wild animals. Many farmers commented on wildlife conflicts between their animals, including dogs, and either predators like lions, hyaenas etc or elephants and hippos. *Kraals* are fenced by either living plants/bushes or by cut wooden palisades and branches. The housing for poultry is usually made of grass. In modern houses, goats have a kraal that is built slightly above the ground so that the manure will fall down. This reduces the risk of parasitic and claw diseases. Pigs are usually kept inside over night, but roam freely during the day. Ectoparasites such as lice and ticks were often found in their surroundings.

2.7.2.2 Animal production for food

Lack of protein is a major nutritional problem in the area. Traditionally, this was addressed by subsistence hunting, but this is not allowed without a license and these are usually too costly for the local people. Many locals therefore eat rats, see Text box 2-2.

Text box 2-2 How to catch a rat

Rats are a delicacy in the study area. I was told, one of the reasons why there are regularly fires around is because this makes it easier to find the rats. To actually get them on your plate, you have to prepare a trap in the evening.


Things needed: a bucket of water, a bowl of maize meal, a hoe and woman power! The next step is to look for paths that the rats have left in the field, for the trained eye an easy job. Then one needs to dig a hole somewhere along the path.


The bucket with water is put into the hole and the maize meal is distributed along the rim to bait the rats. Very important, some maize meal has to be put onto the surface of the water, so that the moonlight won't be reflected by the water and the rats will detect the trap. The next morning one can go to harvest from usually several traps set up.

The rats are roasted on a fire and eaten entirely with head, fur and tail. Bon appétit!

Animals kept as livestock are sometimes eaten, but this is a rare event. It mostly occurs when there is a ceremony like a marriage for instance. When money is needed the animal is sold to a butcher. If an animal is killed unintentionally, through an accident for instance, then the meat is sold around the area. In Figure 2-19 one can see a trader selling pork.

Figure 2-19 A trader who transported pork on his bike to sell it in the area

Butchers can go far to get the animals they need. The slaughtering and selling usually happens in a populated place like Mfuwe where it is easier to market the product with more people and the safari lodges around. However, a butcher is not always called to slaughter an animal as will be explained later.

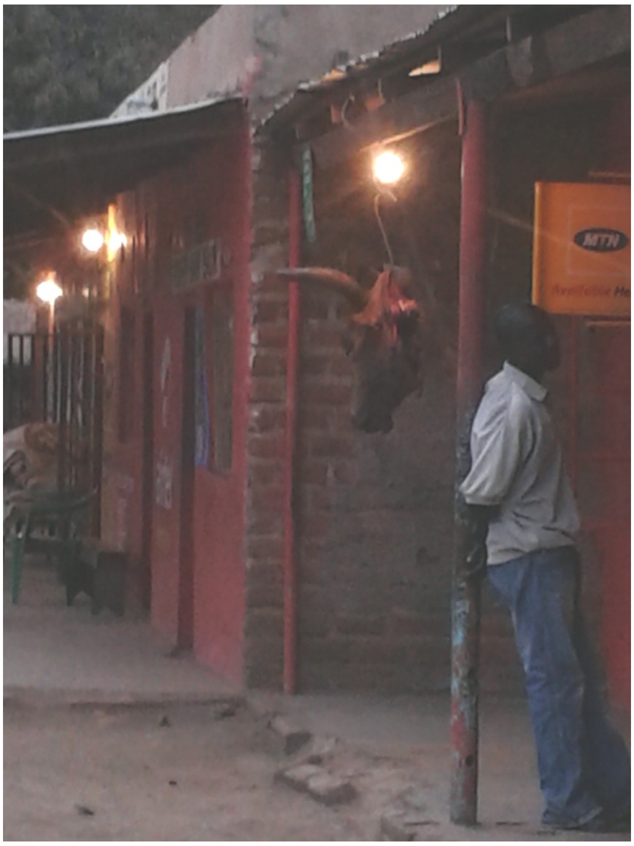


Figure 2-20 Cattle head hanging in front of a shop in Mfuwe

A cattle head hanging in front of a shop means that there is fresh meat available to sell.

2.8 Availability of veterinary services and drugs

The provincial veterinary office is in Chipata and the district veterinary office is situated in Mambwe. There are several people with different expertise and responsibilities belonging to these offices, such as animal production, animal health and vector control.

The study area has only two veterinary surgeons, one is the district veterinary officer and the other one works for the South Luangwa Conservation Society (SLCS), an NGO engaged in anti-poaching activities. The first one is charged with more administrative and livestock population tasks, whereas the latter is dealing with animals that survived snaring or other poaching activities. There is no designated veterinary surgeon in charge of the individual sick animals or sick herds in the study area but Kakumbi tsetse research station can deal with basic veterinary requests as can the tsetse control assistants in Msoro and Kasamanda. Nevertheless, transport is a problem and these people however committed, are not trained in general animal health. Living in the area, they might have some experience of how to deal with certain problems, but since they are also not allowed to possess or sell any drugs, they can be only of limited help to the farmers. The closest place for the famers to access veterinary drugs is Katete.

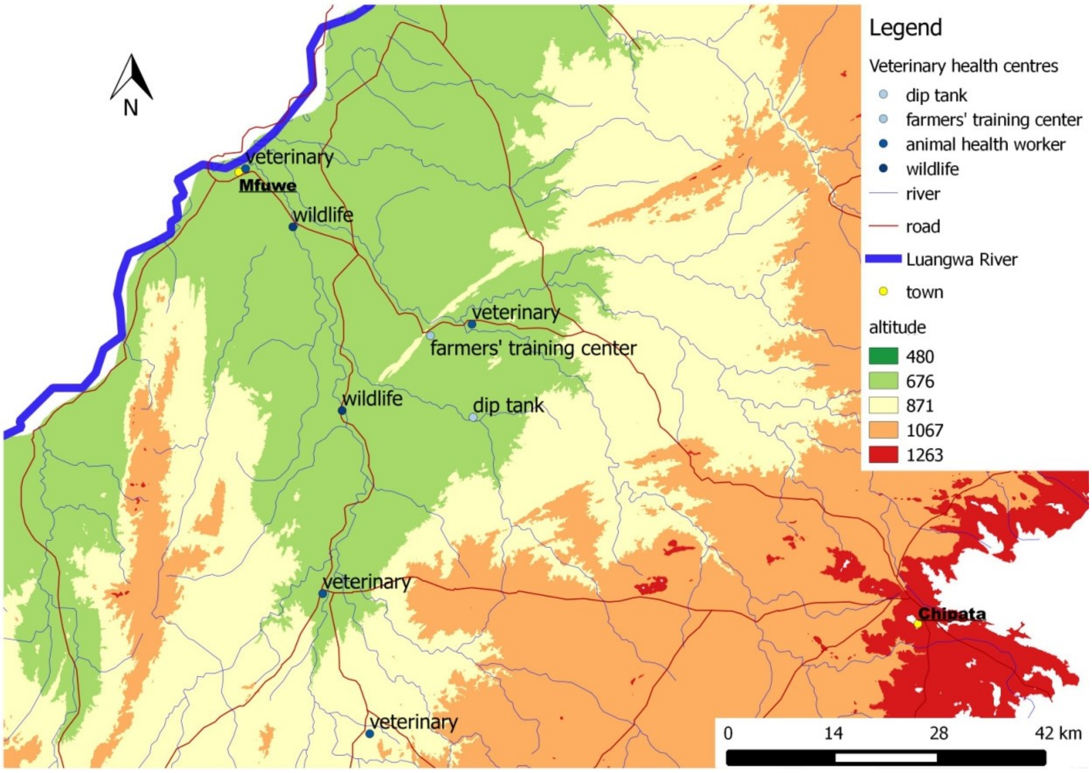


Figure 2-21 Animal health related GPS points within the defined study area

There are five dip tanks in the area for small ruminants constructed under the Adventist Development and Relief Agency (ADRA) about five years ago. ADRA is a global humanitarian organisation of the Seventh-day Adventist Church. They are a very active organisation helping in the development of the area. They are now constructing five dip tanks to accommodate cattle and are about to commission two more to have a total of seven (personal communication Dr N.Machila).

Dip tanks and pour-ons are common measures used to protect cattle against vector-borne diseases, but it has a negative impact on the ecosystem (FAO).

2.9 Tourism

The Zambian tourist industry is not very well developed but has seen increasing growth rates in the 2000s. The increase was higher in Zambia than in the surrounding countries that have more developed tourism sectors. In 2003, 577,000 tourists visited the country, which represented an average annual growth rate of over 11% since 1996. Tourism supported close to 14,000 jobs in the sector and achieved US$149 million in direct revenue, which equals 2.8% of the gross domestic product (GDP). However, the tourism sector is not managed efficiently and has been responsible for uncontrolled growth in urban development in the Mfuwe area (Pope, 2005).

The Luangwa Safari Association (LSA) is a group of 13 safari operators in the area. They manage most tourism related activities and resources and support the community with selected projects. There are 27 camps and lodges in the SLNP that all have more basic and non-permanent bush camps for the authentic safari experience (Nyirenda *et al.*, 2008). The size of the lodges and camps is limited and they should be at least 5 km distance from each other (ZAWA, 2011).

The high season for visitors is between May and October (Pope, 2005). In 2004, 25,000 entries to the national parks were recorded with around 7,000 national tourists and 18,000 international tourists. It is interesting to note that although the growth rate of domestic tourism nearly doubled, overall there were still more international tourists.

All tourist activities are concentrated around Mfuwe. Only 10% of the national park area is used for tourism that results in a low density <0.1 tourist/km^2. There is no access of tourists to the study area, but it has been considered to include the GMA as additional space for consumptive and non-consumptive hunting tourism (Pope, 2005). A new road that connects the Great East Road from Chipata with Mfuwe facilitates the trade with the area and has improved the infrastructure for the tourism industry (Pope, 2005).

2.9.1 Conservation efforts

The great founder of conservation in the Luangwa Valley is Norman Carr, who is still praised today for his efforts. His idea was to combine conservation with tourism to utilise funds from touristic activities for the preservation of the wild animals (Carr, 1996). His approach was new and very modern at his time, but has been taken up around the world as a mean to involve people and increase their interest in the maintenance of wildlife. He himself was a safari guide and tour operator. However, Boyd et al argue that the cost of living with wildlife cannot be compensated by any income from wildlife (Boyd *et al.*, 1999)

The Wildlife conservation society (WCS) wants to improve conservation not through income but through knowledge. They foster science and education on wildlife especially for school children. WCS is located next to the Luangwa River, but a bit further away from Kakumbi and the entrance to the SLNP. It has the space, infrastructure and experience to host and bring up orphans of several wildlife species such as elephants and hippos. They also funded Community Markets for Conservation (COMACO).

Another important stakeholder in conservation is the South Luangwa Conservation Society (SLCS). They support the Zambian wildlife authority (ZAWA) in anti-poaching activities and solving problems of HWC.

Last but not least, there are the community resources boards (CRBs). They are responsible for distributing the wildlife revenues to the community.

2.9.2 Hunting tourism, subsistence hunting and commercial poaching

Hunting is a long-standing tradition of the local population for the purpose of meat consumption. Internationally, it is mainly seen as a sport to have something to add to the trophy collection at home. Since the motivations for hunting are different, Zambia has five different animal quotas in use; for the Minister, research, game capture, traditional ceremonies and problem animals. Everybody who wants to hunt a specific animal species needs a hunting license.

There are five types of hunting licenses in Zambia that are priced on the availability of animal quotas; safari hunting at international safari rates, national hunting for those resident in Zambia, district hunting for those resident in the GMA, special hunting licenses for research or special traditional occasions. And finally there is illegal subsistence or commercial poaching (Pope, 2005). Hunting can also be classified into consumptive and non-consumptive hunting. Consumptive hunting includes the killing of an animal whereas non-consumptive hunting is done by watching, photographing or studying wildlife. Both can have negative impacts on the animal population as the former one directly reduces the animal population while the latter creates disturbances.

As mentioned earlier, traditionally the inhabitants of the valley lived by subsistence hunting. Nowadays, hunting is only allowed if a license is bought for the animal to be hunted. These licenses, although cheaper for Zambians, are usually not affordable for the valley people.

Another conflict that Pope mentions is the fact that district licenses are far more subsidised than national licenses with a value below the market price of the hunted meat, but those are the type of license mainly granted (Pope, 2005). Both together represent 90% of the total animal quota, but contribute only 10% of the total hunting revenue from ZAWA (Pope, 2005). In consequence, this licensing system neither improves the economy of the study area through the community resource boards (CRBs) nor does it alleviate the lack of protein that inhabitants of the GMA are threatened with.

Additionally, increasing urban demand for bush meat aggravates the development from subsistence poaching to commercial poaching. Poaching of game is on the rise, it is a big business. Middlemen are now in charge of the trade not only of bush meat but also ivory, hippopotamus teeth, hides and lion and leopard skins. Most of the ivory is exported to Asia for use in Chinese traditional medicine and other purposes (Gao and Clark, 2014).

Or as Carr says;

> "Anyone with enough political clout, or who has a friend in court, or enough cash, can get a license irrespective of the set quotas." (Carr, 1996)

It had been suggested this licensing system be abolished and one created that encourages locals to participate in the conservation and efficient management of their wildlife resources. However, the idea has not yet been implemented and the presumptive misuse by safari outfitters, traditional leaders, politicians and the wildlife management organisation itself together with a general lack of transparency has had deleterious effects. In fact, ZAWA had inconsistencies in their records that suggested misuse and abuse of resources to the extent that the ZAWA scouts had the reputation for having a hands-off approach with poachers if not trading in bush meat themselves (Pope, 2005). Experience shows that committed ZAWA scouts are needed to inhibit poaching activities (Dalal-Clayton *et al.*, 2003).

The financial benefits from hunting tourism are extremely low in Zambia compared to other countries; South Africa and Zimbabwe earn US$ 16/ha, Botswana and Namibia around US$ 8/ha and Zambia less than US$ 1/ha.

The revenues gained from hunting activities in the Lupande GMA contribute to the funds of ZAWA and the community resource boards (CRBs) in the area. Although this income has a significant positive impact on the economy and sociology of the area in that it provides employment, hence a growth in skilled personnel and a better infrastructure, it also causes a lot of problems. Pope mentions as part of the negative impact an increase in HIV/AIDS prevalence, delinquent behaviour, unplanned urban development, deforestation, illegal hunting and fishing (Pope, 2005). The local government therefore has to take more charge in administrating and managing the requirements needed to reduce the problems arising from an increasing tourism and hunting sector in the area (Pope, 2005).

Fish farming, husbandry of wild species and game ranching are suggested as solutions to reduce the need for poaching for local people (Carr, 1996; Pope, 2005).

In summary, the intention of several projects including the Luangwa Integrated Resource Development Project (LIRDP), a major project with various aims conducted from 1986-2002, has been to generate money from wildlife for the benefit of the community. Unfortunately, the administrative bodies and organisations in charge abused/misused the generated funds which resulted in a loss of trust and respect by the local community (Dalal-Clayton and Child, 2003; Pope, 2005).

Furthermore a number of HWCs occur in the area. The general public has been forbidden to possess and use firearms since the 19th century, because colonials were concerned with conservation issues, although they themselves implemented trophy hunting safaris. The law is in place until today and thus inhabitants cannot protect themselves against the animal attacks. They are supposed to be protected by ZAWA, but the latter cannot provide sufficient service due to lack of staff, transport and adequate equipment (Marks and Fuller, 2008). The government also does not compensate for any loss, be it livestock or human. Poaching is important for the inhabitants as a protein source but increasingly over the past decades also for generating cash. The increased law enforcement against possession of firearms reduced poaching of big species by locals but led to greater use of snares on smaller species that can easily vanish unnoticed (Dalal-Clayton *et al.*, 2003)

There are usually only a few people in the community that are hunters, most are carriers or spotters. They train dogs and risk the use of illegal firearms which are also supposed to protect them from further animal attacks (Marks and Fuller, 2008).

Nowadays, poaching wildlife is a big business; more poachers are coming from outside the region in large well organised groups in one night, kill many animals and bring dead animals up to the plateau immediately. The poachers sell their haul from the plateau directly; the meat goes to Lusaka and the ivory, hides etc are transported to Asia or elsewhere, probably via South Africa. ZAWA scouts may find these people, but are too scared to face them because of above mentioned deficiencies. The scouts also feel let down by the authorities, being unpaid for long periods, and therefore might even become involved in illegal activities.

In Zimbabwe, poachers have gone so far to use cyanide or aldicarb to kill elephants, which usually sweeps out a whole elephant family, having tusks or not. This method also kills other animals arbitrarily and contaminates the water and ground (ProMED-mail, 2013c).

These complex interrelations have an influence on conservation and biodiversity, the transmission of diseases through closer contact with wildlife, livestock production, the socio-economic status of a household and a reduced wellbeing and livelihood of local people.

Chapter 3: Survey on the disease status in animals

This chapter focuses on the animal health component of the study conducted in Mambwe District, Eastern Province, Zambia. Samples were collected between June and August 2013. The study describes the infections tested for in livestock (cattle, sheep, goats and pigs) and dogs and the prevalence found, the methods applied to study them and the conclusions realised. The study included blood sampling in the field, diagnosis in the field and in the laboratory and animal health questionnaires.

3.1 Introduction

Apart from the infections this study focuses on, there are a number of other infectious diseases that play a role in animal health in Zambia. There are continuous outbreaks of anthrax in livestock and people, mainly in the Western Province. Very often farmers acquire anthrax infections through the consumption of a deceased animal (ProMED-mail, 2013b; ProMED-mail, 2015b). Outbreaks of peste des petits ruminants has been reported in Northern, Eastern, North-Western and Copperbelt Provinces in 2015 (ProMED-mail, 2015f). The economically important lumpy skin disease has existed in Zambia since 1929 and there was a recent outbreak reported in Muchinga Province in 2015 (ProMED-mail, 2015e). Foot and mouth disease is another notifiable disease occurring in Zambia with a reported outbreak in Western Province in 2015 (ProMED-mail, 2015d). Another disease often reported as a problem in Zambia is rabies. There were on average 2.93 dog rabies cases per month in Zambia during 1985-2004. The occurrence of dog bites is mainly related to human population density and the possibility of a person being bitten to have access to a medical facility (Munang'andu *et al.*, 2011). Tuberculosis is also a big problem in some parts of Zambia at the wildlife-livestock-human interface (Malama *et al.*, 2013). Recently, African wild dogs have been found positive for bovine tuberculosis which could have devastating effects on the already threatened population (ProMED-mail, 2016a).

The following diseases were chosen and these were dependent on the knowledge and resources available to conduct the survey; brucellosis, African swine fever, tick-borne pathogens, trypanosomiasis and cysticercosis. African swine fever was of great concern to the Zambian government which is why it was included. Brucellosis, trypanosomiasis and tick-borne pathogens were well established research topics within the team and diagnostic capacities were available without a problem. Cysticercosis was known to be a problem in neighbouring areas and therefore questions in relation to this disease were included.

3.1.1 Aims of the chapter

The main objective was to identify the problems farmers are having to deal with in their rearing of livestock and dogs. The aims of the chapter are to present the prevalence of selected animal diseases during the time frame of the study. It also looks at the contributing factors such as body condition score and reproductive status and lists the signs observed in the individual animal and in conclusion the results will be used to identify any changes or developments observed that will be compared to results of previous studies sourced from available literature.

3.1.2 Trypanosomiasis in the study area

Trypanosomiasis is an endemic disease in the Luangwa valley (Mubamba *et al.*, 2011) where ecological and climatic conditions favour the tsetse fly, the vector of African trypanosomiasis (Mubamba *et al.*, 2011). Tsetse flies have feeding preferences which are reflected in their parasite-host relationships (Clausen *et al.*, 1998). Infection is caused by different blood parasites; the zoonotic *T. brucei rhodesiense* in eastern and southern Africa and *T. brucei gambiense* in western

Africa with a less certain role as zoonosis (Welburn *et al.*, 2001; Welburn and Maudlin, 2012), whereas *T. brucei brucei* does not infect humans (Barrett *et al.*, 2003b). *T. vivax* is known to be transmitted by tabanids and stable flies as well as the tsetse fly (Desquesnes and Dia, 2003; Torr *et al.*, 2006). *T. congolense* can be savannah type, riverine-forest or Kilifi and is most frequently found in cattle (Masumu *et al.*, 2012; Simukoko *et al.*, 2007a; Sinyangwe *et al.*, 2004). In rare cases, it can be transmitted by tabanids too (Desquesnes and Dia, 2003). In Eastern Province, only the savannah type was found (Masumu *et al.*, 2006a; Masumu *et al.*, 2012). *T. simiae* is highly pathogenic in pigs (Auty *et al.*, 2012; Gibson *et al.*, 2001; Woolhouse *et al.*, 1996). *T. godfreyi* infects mainly warthogs and other wild and domestic suids (Auty *et al.*, 2012; McNamara *et al.*, 1994).

Trypanosomiasis is considered to be endemic with lower morbidity and mortality when cattle are both hosts and reservoirs, but the dynamic is altered and the disease becomes epidemic with higher morbidity and mortality when wildlife is involved as a reservoir (Van den Bossche and Delespaux, 2011). The closer contact between people, livestock and wildlife changes the whole epidemiologic pattern of trypanosomiasis (Van den Bossche *et al.*, 2010). *Trypanosoma* species have been identified from several wildlife species in the Luangwa valley (Anderson *et al.*, 2011; Auty *et al.*, 2012) and additionally can infect all domesticated livestock species (Ng'ayo *et al.*, 2005).

Figure 3-1 Relict of the historical importance of trypanosomiasis in the area

High protein intake can reduce the effect of trypanosomiasis in animals infected with *T. brucei brucei* (Nnadi *et al.*, 2010). It is therefore important to provide high quality feed to animals at risk or infected with African *Trypanosoma {Holmes, 2000 #1788}{Nnadi, 2010 #1789}*.

Trypanosomiasis prevalence in cattle from Eastern Province decreased steadily between 1989-2007 from 17.31% to 1.16% (Mubamba *et al.*, 2011). This was mainly achieved by vector control and treatment of animals. The mortality due to trypanosomiasis has reduced as well during the same time (Mubamba *et al.*, 2011). More recently (2006-2008), prevalence has increased in Lundazi district for unknown reasons (Mubamba *et al.*, 2011). There was a peak of trypanosomiasis cases in cattle between 1990-1992 with a prevalence of 50% in Katete and Petauke district (Mubamba *et al.*, 2011). The highest prevalence has been observed in Mambwe district which has the greatest density of wildlife in the province (Mubamba *et al.*, 2011). However, there is a high variation in prevalence within one area due to tsetse fly habitat fragmentation (Ducheyne *et al.*, 2009; Masumu *et al.*, 2012). Although the prevalence has been high, there was a low economic impact on livestock production (Doran, 2000; Masumu *et al.*, 2012). This is probably because the strains showed a low virulence and there was cross-protection between low and highly virulent strains (Masumu *et al.*, 2012). Most strains in livestock are of low virulence (Bengaly *et al.*, 2002; Masumu *et al.*, 2012). They have an effect on reproduction, but do not cause death (Masumu *et al.*, 2012). More virulent strains are more effectively transmitted (Masumu *et al.*, 2006b; Masumu *et al.*, 2012), but they are more likely to kill the host hence highly virulent strains tend to be eliminated and those of lower virulence become the predominant infecting trypanosomes (Masumu *et al.*, 2006a; Masumu *et al.*, 2012).

Data from 2005 identified *T. congolense spp*., *T. vivax*, *T. brucei spp*. and *T. theileri* in Mambwe district with following species prevalence: cattle 33.3% (95% CI 29.66-37.05), goats 10.2% (95% CI 8.23-12.53), sheep 27.6% (95% CI 16.66-40.9), pigs 20.9% (95% CI 15.17-27.64) (Mubanga, 2008).

A similar pattern with *T. vivax* being the most frequent parasite has also been reported from Burkina Faso (Silbermayr *et al.*, 2013) where indigenous taurine cattle breeds were found to be trypanotolerant (Silbermayr *et al.*, 2013).

Figure 3-2 Dip tank in the area

Cattle in trypanosomiasis infested areas have in general lower calving rates, lower milk yields and higher rates of calf mortality and thus create a severe economic burden to Africa's livestock agriculture (Swallow, 2000). The costs for producers and consumers are an estimated USD 1340 million annually without taking into account the impact upon traction and manure (Kristjanson *et al.*, 1999). Antibody seroprevalence is a reliable measure to predict the average annual parasitological prevalence (Machila *et al.*, 2001). There are two drugs available against African animal trypanosomiasis, isometamidium chloride (Samorin® or Trypamidium®) for prevention and diminazene aceturate (Berenil® or Veriben®) for curative treatment (Van den Bossche *et al.*, 2000). Diminazene aceturate is also active against babesiosis and some *Theileria* species (Van den Bossche *et al.*, 2000). Oxen and cows are most frequently treated, probably because they are more likely to be infected (Masumu *et al.*, 2012; Simukoko *et al.*, 2007a; Simukoko *et al.*, 2007b; Van den Bossche *et al.*, 2000). The Eastern Province shows a spread of drug resistance on the plateau due to genetic exchange between resistant and susceptible trypanosome strains (Delespaux *et al.*, 2008; Masumu *et al.*, 2012; Sinyangwe *et al.*, 2004; Van den Bossche *et al.*, 2000). In Mambwe district, mainly sick animals are treated and this probably increases resistance but decreases mortality (Masumu *et al.*, 2012).

Figure 3-3 A typical model of a trap to catch tsetse flies

Dogs can become infected with trypanosomes, but there is not much literature about it. Abenga et al experimentally infected six puppies with *T. congolense* in Nigeria (Abenga *et al.*, 2004). Although the dogs showed parasitaemia after 6 to 7 days, they showed no signs of disease from which they concluded there was a degree trypanotolerance in dogs (Abenga *et al.*, 2004). Exotic dog breeds showed infection with *T. congolense* and *T. brucei rhodesiense* in the Luangwa and Zambezi Valley (Namangala *et al.*, 2013). Similar results were reported from Kenya with corneal opacity as predominant clinical sign (Matete, 2003). A study on 237 dogs from Mambwe district using LAMP PCR showed a prevalence of 5.9% and the infections were caused by *T. congolense*, *T. brucei brucei* and *T. brucei rhodesiense* (Lisulo *et al.*, 2014).

Natural infection of feline species with trypanosomes has been shown only in wildlife (Anderson *et al.*, 2011). Experimental infection of domesticated cats (*Felis sp.*) with *T. evansi* produced fever, facial inflammation and loss of appetite at intervals of 2 weeks and the authors claimed to show the presence of amastigote, sphaeromastigote and trypomastigote forms of *T. evansi* in the brain of infected cats (Choudhury and Misra, 1972; Choudhury and Misra, 1973).

3.1.3 Tick-borne diseases in cattle and dogs in the study area

The importance of a number of tick-borne pathogens (TBP) from the genera *Theileria*, *Anaplasma*, *Babesia*, *Ehrlichia* and *Rickettsia* was also determined. There has been major renaming and re-classification of the various genera due to improvement of molecular identification methods. Although the various pathogens are transmitted by different species of tick vectors, this was not taken into account in this study. Some *Rickettsia* (*R. typhi, R. felis, R. prowazekii*) might even be transmitted by other, non-tick vectors such as fleas and lice (Parola, 2006; ProMED-mail, 2015h). At least one publication mentions tick-borne diseases (TBDs) as the main constraint for livestock production in Mambwe district (ZAWA, 2011). The burden of ticks is significantly higher in wet than in dry season (Berkvens *et al.*, 1998; Simuunza *et al.*, 2011). TBDs in dogs are considered a growing threat (Chomel, 2011). Tourists visiting game areas have a significantly higher risk of contracting a TBI due to high infection rates in wildlife (Chitanga *et al.*, 2014; Oura *et al.*, 2011). Because of the zoonotic nature of most TBDs, the use of the One Health approach to control these diseases is recommended intervening at the human, animal and ecosystem level (Dantas-Torres *et al.*, 2012).

Among the genus *Theileria,* this study investigated *Th. annulata*, *Th. buffeli*, *Th. equi*, *Th. equi-like*, *Th. mutans*, *Th. parva*, *Th. taurotragi*, *Th. velifera*, *Th. sp* MSD4 and *Th. sp* (duiker). There are many reports of *Th. parva* in cattle, since it is the cause of the highly pathogenic East Coast fever (ECF) that is common in eastern and southern Africa (Mubamba *et al.*, 2011). However, there are reports of subclinical carriers in cattle which would contribute significantly to the spread of the disease, not only African buffaloes that are usually implicated (Yusufmia *et al.*, 2010). A retrospective epidemiological study analysing reports on ECF in Eastern Province from the past 20 years identified a decreasing prevalence of 1-6% from 1989-1994. Immunization programmes led to a prevalence of 1-3% from 1993-2000. A further decrease of 0.1-0.5% from 2001-2008 has been reached using anti-*Theileria* drugs (Mubamba *et al.*, 2011). However, there are a few cases of ECF in the valleys of

Eastern Province which could be related to relatively low populations of cattle or to lower density of the suitable vector because of climatic conditions (Mubamba *et al.*, 2011). The overall mortality due to TBDs also decreased over the years (Mubamba *et al.*, 2011). In 2007/2008, Simuunza et al. found a prevalence of *Th. parva* on the plateau of 22% and 30.3% for the dry and wet season respectively using PCR (Simuunza *et al.*, 2011). The same study identified a prevalence of *Th. taurotragi* of 21.3% and 36.5% and *Th. mutans* of 48.8% and 83.9% in dry and wet season respectively (Simuunza *et al.*, 2011). *Th. mutans*, *Th. taurotragi* and *Th. velifera* are currently considered apathogenic/ low pathogenic species, but seem to play a big role as co-infections with protective effects (Jongejan *et al.*, 1986; Musisi *et al.*, 1984; Simuunza *et al.*, 2011). Theileriosis in dogs causes anaemia and thrombocytopenia with bleeding and lethargy as signs (Rosa *et al.*, 2014). The responsible pathogens amongst others can be *Th. equi*, *Th. annae* and *Th. annulata* (Rosa *et al.*, 2014).

For *Anaplasma*, this study investigated *A. bovis*, *A. centrale*, *A. marginale, A. phagocytophilum* (formerly *Ehrlichia equi, Ehrlichia phagocytophila*, and human granulocytic Ehrlichial agent) and *A. platys (*formerly *Ehrlichia platys)*. Anaplasmosis caused by *A. marginale* is a disease with a major impact on animal health in sub-Saharan Africa (Simuunza *et al.*, 2011). In 2007/2008, a prevalence of *Anaplasma spp*. in Katete and Petauke districts of 40.8% and 63.5% was found in dry and wet season respectively (Simuunza *et al.*, 2011). A species specific PCR targeting the 18S rRNA gene and 16S rRNA gene was used. Recently, a new *Anaplasma* species has been identified in goats in China and named *A. capra* (ProMED-mail, 2015a). To investigate if the pathogen is zoonotic, the researchers tested patients with a history of a tick bite and found many positives (ProMED-mail, 2015a). This increases the potential pathogens for fever of unknown origins. *A. phagocytophilum* is zoonotic although there have been no confirmed human cases in southern Africa yet reported (Chitanga *et al.*, 2014). It is known to occur in dogs (Chitanga *et al.*, 2014). Anaplasmosis has also been reported in wildlife in the Eastern Province (Makala *et al.*, 2003).

E. ruminantium, *E. sp omatjenne*, *E. chaffeensis* (mostly a human infection) and *E. canis* (mostly a dog infection) were tested for the genus *Ehrlichia*. *E. ruminantium* causes heartwater. This disease can lead to high mortalities in animals in Africa and the Americas, e.g. camels, and is considered zoonotic (Chitanga *et al.*, 2014; ProMED-mail, 2013d; Simuunza *et al.*, 2011). On the plateau of the Eastern Province, *E. ruminantium* was present in cattle at 5.4% and 19% in dry and rainy season in 2007/2008 respectively (Simuunza *et al.*, 2011). *E. chaffeensis* is zoonotic and probably *E. canis* too (Chitanga *et al.*, 2014).

As part of the *Babesia* genus, *B. bigemina*, *B. bovis*, *B. caballi*, *B. divergens*, *B. canis canis*, *B. canis vogeli*, *B. rossi* and *B. major* were all tested for. Babesiosis is a disease caused by *B. bigemina* and *B. bovis* with high impact on livestock in sub-Saharan Africa (Simuunza *et al.*, 2011). The prevalence in cattle on the plateau of Eastern Province was in 2007/2008 for *B. bovis* 10.9% and 32.7% and for *B. bigemina* 0.2% and 11.8% during dry and wet season respectively (Simuunza *et al.*, 2011). Babesiosis has also been reported in dogs in Zambia (Makala *et al.*, 2003). Toy breeds in South Africa have a lower risk of babesiosis than indigenous breeds (Mellanby *et al.*, 2011). *B. bovis* and *B. divergens* are zoonotic although there were only two reports of human babesiosis in South Africa so far (Bush *et al.*, 1990; Chitanga *et al.*, 2014).

Finally, the genus *Rickettsia* was represented by *R. conorii*, *R. helvetica*, *R. massiliae* and *R. sp* (DnS14)/*raoultii*. All these microbes are zoonotic in addition to *R. africae*. *R. africae* causing African tick bite fever has been found several times in travellers to Africa. It usually presents with mild infections (Raoult *et al.*, 2001). *Rickettsia* microbes have not yet been reported in humans in Zambia, but have been in neighbouring countries including Zimbabwe and Kenya (Cazorla *et al.*, 2008; Parola, 2006).

Simuunza *et al.* published the prevalence of several TBP from blood samples taken in 2007 and 2008 in Petauke and Katete districts (Simuunza *et al.*, 2011). Interestingly, the prevalence was always lower in the dry season than in the rainy season. This was not the case with the samples from other provinces (Simuunza *et al.*, 2011). This could be due to more regular treatment with long-acting tetracycline when the risk from TBD is high (Simuunza *et al.*, 2011).

The impact on productivity of single or multiple pathogen forms of TBD are not well known, but with TBP high numbers of mixed infections in animals are observed (Ait Lbacha *et al.*, 2015; Georges *et al.*, 2001; Gubbels *et al.*, 1999; Martins *et al.*, 2010; Rosa *et al.*, 2014; Simuunza *et al.*, 2011). Also, ticks can carry multiple pathogens (Christova *et al.*, 2003). Some co-infections seem to support the development of endemic stability of TBD in cattle (Jonsson *et al.*, 2012). Calves face a high chance of becoming infected by ticks, but they do not show clinical signs (Jonsson *et al.*, 2012). Adult cattle have then a high level of immunity and consequently low incidence of clinical disease (Jonsson *et al.*, 2012). This is especially the case with *Th. parva* and low pathogenic *Theileria* (LPT) species such as *Th. mutans* and *Th. velifera* (Woolhouse *et al.*, 2015). The presence of LPT in calves leads to a net decrease in mortality due to ECF of 43% (Woolhouse *et al.*, 2015). Simultaneously, the growth rate of calves is higher when infected with *Th. parva* and *Th. mutans* as compared to *Th. parva* infection alone (Thumbi *et al.*, 2013). Buffaloes show a high prevalence and diversity of LPT and this might be the reason why they are so resistant to *Th. parva* (Oura *et al.*, 2011; Woolhouse *et al.*, 2015). In contrast, concurrent infection of *Th. parva* and a *Trypanosoma* species increases the risk for ECF death by six times (Thumbi *et al.*, 2014). Strongyle infection also raises mortality (Thumbi *et al.*, 2014).

3.1.4 Brucellosis in the study area

Brucella are gram-negative, intracellular bacteria in the shape of cocci, coccobacilli or short rods (Coetzer and Tustin, 2004a). They are the cause of the world’s most common bacterial zoonosis (Ducrotoy *et al.*, 2016). They infect the genital organs and cells of the monocyte-macrophage series in wild and domestic mammals and are transmitted by foetal membranes and fluids including vaginal discharges, milk and semen. The zoonotic species of *Brucella* are *Br. melitensis*, *Br. abortus, Br. suis* and *Br. canis*, whereas *Br. ovis, Br. neotomae* and *Br. pinnipediae* are host species specific (Coetzer and Tustin, 2004a; Smirnova *et al.*, 2013). Co-infections of two different *Brucella* species have not been documented and are unlikely due to cross-immunity between *Brucella* species (Ducrotoy *et al.*, 2015). Signs in animals are usually abortions or reproductive disorders and hygroma. In humans, it causes fever, chills, depression, weakness, headache, joint pain, generalised aches and sweating (Coetzer and Tustin, 2004a). *Br. abortus* is more often found in cattle compared to *Br. melitensis* that is found mainly in small ruminants or camels. Also, dogs and wildlife have been shown infected with the latter. Some sheep breeds can show resistance to it (Coetzer and Tustin, 2004a).

A characteristic feature of the organism that is used in the diagnosis of *Brucella* species is their occurrence as a smooth form, while others present in a rough form. This is defined by smooth and rough lipopolysaccharides (S-LPS and R-LPS) (Coetzer and Tustin, 2004a). There are many serological tests available to diagnose past infection, present infection and carrier state by the type and amount of antibodies found, but none of them is perfect (Abernethy *et al.*, 2012; Coetzer and Tustin, 2004a; Diaz-Aparicio *et al.*, 1994; Ducrotoy *et al.*, 2015; Ducrotoy *et al.*, 2016; Godfroid, 2002; Huber and Nicoletti, 1986; Muma *et al.*, 2009; Muma *et al.*, 2007c; Nicoletti, 1992; Smirnova *et al.*, 2013). These tests have also been applied to diagnosis in humans (Diaz *et al.*, 2011; Smirnova *et al.*, 2013). Sometimes only multiple testing will reveal positives (Abernethy *et al.*, 2012). The Rose Bengal test (RBT) detects IgM and IgG antibodies only of Brucella species containing S-LPS. It is known to yield many false positive results but have a good specificity. The sensitivity of RBT depends mainly on the quality of the antigen (personal communication Ignacio Moriyon, (Blasco *et al.*, 1994)), but is also related to its cross-reactivity with *Yersinia enterocolitica* (Smirnova *et al.*, 2013). The gold standard and only way of species identification is bacterial culture. However, it is not easy to perform and this technique is only rarely applied (Ducrotoy *et al.*, 2016).

The use of antibiotics such as penicillin and oxytetracycline makes the *Brucella* spp. undergo L-transformation (without a cell wall) and is thus no longer detectable using serological techniques, resulting in an increasing number of carrier animals (Banai *et al.*, 2002; Brucellosis, 1986).

A common prevention method is vaccination and this has been used in Zambia (Ducrotoy *et al.*, 2015; Gallagher, 1973; Schuurman, 1983). The risk of a spread of the vaccine strain in wildlife is high (Godfroid, 2002). Also, diagnosing active brucellosis in a vaccinated population may prove difficult (Muma *et al.*, 2012).

Since contact between domesticated livestock and wildlife is considered to be a risk in the spread of both human and animal brucellosis in Zambia, it was integrated into this study (Coetzer and Tustin, 2004a; Godfroid, 2002; Muma *et al.*, 2007a; Muma *et al.*, 2007b; Muma *et al.*, 2006; Munang'andu *et al.*, 2006). Buffaloes (*Syncerus caffer*) were found positive for brucellosis and Kafue lechwe (*Kobus leche kafuensis*) with a prevalence of 21.6% in 2003-2008 and 42.9% in 2009 in the Kafue flats in Zambia (Muma *et al.*, 2010; Muma *et al.*, 2011). The Black lechwe (*Kobus leche smithemani*) from the Bangweulu swamps had no brucellosis detected in 2009 and it was hypothesized that this was due to the lack of contact with livestock (Muma *et al.*, 2011).

In 2007, Chimana *et al.* conducted a cross-sectional study on brucellosis on commercial farms in Lusaka province and on rural farms in Central Province. They tested a total of 897 sera from cattle using RBT and found 7.9% seroprevalence on the commercial farms and 18.7% for the rural farms (Chimana *et al.*, 2010). Similar results were obtained in Southern Province of Zambia with traditional farms having higher prevalence (Muma *et al.*, 2013). These results would support the theory that commercial farmers are more knowledgeable of the likely animal health issues in raising livestock and invest more in their farming so that the conditions to acquire and maintain *Brucella* infections in a herd are less favourable. However, in Tanzania it was shown that commercial dairy farms were more prone to brucellosis infections (Jiwa *et al.*, 1996).

Several studies detected a brucellosis seroprevalence in cattle in the Kafue flats of 14.1-28.1% and were able to connect it to grazing habits and wildlife contact (Ghirotti *et al.*, 1991; Muma *et al.*, 2006). No goats or sheep tested positive in the same survey (Muma *et al.*, 2006). This might be because the agent is *Br. abortus* and not *Br. melitensis* (Muma *et al.*, 2006). Bovine brucellosis is particularly prevalent in the Western Province of Zambia with up to 30% positivity (Ghirotti *et al.*, 1991).

In the neighbouring country Zimbabwe, dogs were tested for *Br. canis* in urban Harare and five rural communities and a seroprevalence of 12.7% and 20.7% respectively was detected (Chinyoka *et al.*, 2014).

3.1.5 Cysticercosis in the study area

Cysticercosis is a zoonotic parasitic infection with *Taenia solium* involving pigs and *Taenia saginata* involving cattle as intermediate hosts. The lifecycle of both cestodes is very similar, except that metacestodes of *Taenia solium* migrate into neuronal tissues, muscles or eyes and cause neurocysticercosis (NCC) as seen below in step 7-9 (OIE, 2012).

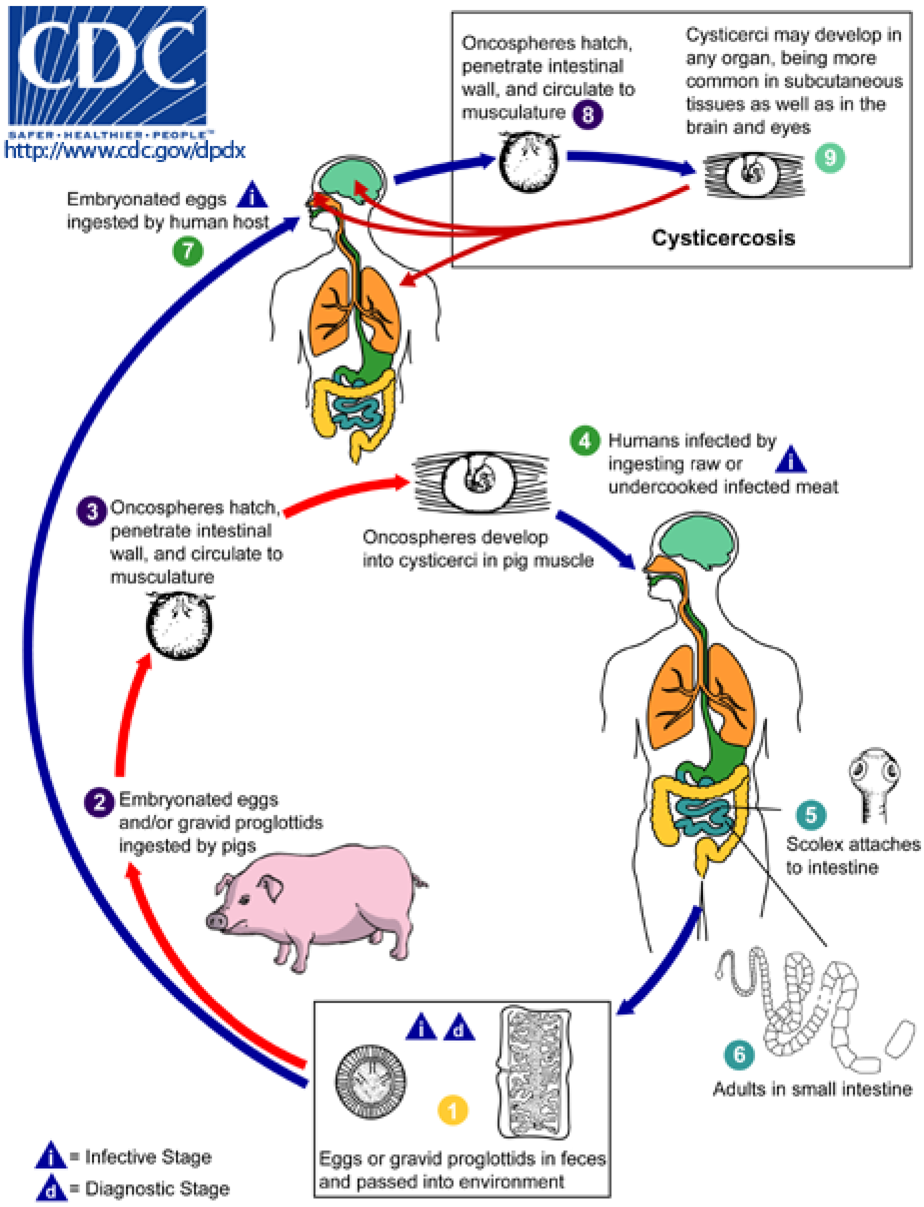


Figure 3-4 Life cycle of *Taenia solium* (© CDC)

NCC is the most frequent cause of epilepsy in developing countries affecting some 50 million people (Assana *et al.*, 2013; Craig and Pawlowski, 2002; Forsgren, 2008; Wu *et al.*, 2013). Diagnosis is difficult in humans and special criteria need to be applied (Del Brutto, 2012). The best treatment is a combination of praziquantel and albendazol. Praziquantel alone at a high dosage can become a diagnostic treatment for suspected cases (Wu *et al.*, 2013). Unfortunately, up to 90% of affected people do not receive appropriate treatment (Forsgren, 2008). Also, people suffering from epilepsy in traditional societies tend to be stigmatized and are socially and economically disadvantaged (Birbeck *et al.*, 2007; Birbeck *et al.*, 2008). In epileptic women, rape rates of 30% were seven times higher than usual (Birbeck *et al.*, 2007).

In Zambia, pork production takes place mainly in two provinces, 60% in Eastern Province and 30% in Southern Province (Wilkinson *et al.*, 1988). Because of the risk of spread of African swine fever, pigs are not allowed to be moved out of Eastern Province (Craig and Pawlowski, 2002).

For the diagnosis of neurocysticercosis, the Del Brutto diagnostic criteria are used, consisting of neuroimaging and antibody detection in serum. However, detection of antigen in serum has shown more accurate results with a sensitivity of 100% and specificity of 84% and should be considered when diagnosing neurocysticercosis (Gabriel *et al.*, 2012).

A study in 2002 looking at the prevalence in pigs using this Ag-ELISA found 20.8% of pigs infected in the Southern Province and 9.3% in Eastern Province (Phiri *et al.*, 2002). This was mainly related to poor knowledge and practices, poor hygiene conditions and absence of meat inspection and control (Phiri *et al.*, 2002). A study in Eastern Province using tongue palpation and Ag-ELISA showed a prevalence based on tongue palpation of 7% and by Ag-ELISA 16.9% (Sikasunge *et al.*, 2008). Crossbred pigs were more likely to be infected (Sikasunge *et al.*, 2008). Identified risk factors for people to become infected in Eastern Province were lack of pork inspection at slaughter (96.7%), consumption of pork with cysts (20.1%), selling of pork infected with *T. solium* cysticerci (18.3%), free-range husbandry system (83.2%) and absence of latrines (58%) (Sikasunge *et al.*, 2007). Interestingly, the pigs at the periphery of the villages were more exposed to tapeworm eggs (Sikasunge *et al.*, 2007). There are similar findings in the eastern and southern African region (Afonso *et al.*, 2011; Phiri *et al.*, 2003; Rottbeck *et al.*, 2013).

Additionally, a study in Eastern and Southern Africa revealed that non-pork eaters (here Xhosa-speaking people) had the same risk of getting infected as pork eaters (Mafojane *et al.*, 2003). In some places, traditional healers are using *Taenia* segments for their treatments (Mafojane *et al.*, 2003). Looking more at the human infection, it has been shown that *T. solium* infections are very dynamic (Mwape *et al.*, 2013b). (Re) infection happens often in a highly contaminated environment, but many infections turn sero-negative within one year (Mwape *et al.*, 2013b). The results of this study indicate that 1 in 3 people are exposed to infection but only 1 in 10 acquires infection (Mwape *et al.*, 2013b). Mwape et al conducted a prevalence study in humans in the Eastern Province (Petauke district) in Zambia and found taeniosis stool prevalence of 6.3% and serum cysticercosis prevalence of 5.8% (Mwape *et al.*, 2012). The prevalence increased significantly in people older than 30 years (Mwape *et al.*, 2012).

Bovine *Taenia saginata* cysticercosis was reported with a prevalence of 6.1% in Central and Southern Province (Dorny *et al.*, 2002).

3.1.6 African swine fever in the study area

African swine fever (ASF) is a haemorrhagic disease of pigs caused by a DNA virus in the family of *Asfarviridae*. It was first reported in 1912 in Eastern Province in Zambia and is considered to be enzootic since in the country. Warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus sp*) and giant forest hogs (*Hylochoerus meinertzhageni*) are a reservoir of the virus. Transmission happens directly, indirectly or through *Ornithodoros* ticks (Coetzer and Tustin, 2004b). Mortality is high in naive populations, causing severe economic losses (Samui *et al.*, 1996), and no treatment or vaccine exists (Coetzer and Tustin, 2004b). Recent outbreaks occurred in Zambia in 2013 and 2014 in Lusaka Province (ProMED-mail, 2013a; ProMED-mail, 2014a). The spread of the disease is easily facilitated in systems where the animals are free roaming. Pigs have found to be travelling more than 4 km in a mean home range of on average 10, 000 m^2 (Thomas *et al.*, 2013). A study in 1988 looking at ASF virus in ticks in Zambia found a prevalence of infection in adult ticks of around 5% (Wilkinson *et al.*, 1988).

3.2 Materials and Methods - Animals

This part of the thesis gives a description of where and how the analysis of this survey was done. It deals with all analysis related to animal health. However, some methods were applied to studies in humans and will be described in a subsequent chapter.

3.2.1 Study design

The study design chosen due to budget constraints is a cross-sectional survey in five animal species; goats, cattle, sheep, pigs and dogs. In a cross sectional survey only point prevalence can be estimated. It was decided to conduct a one stage cluster sampling with the cluster defined as one household. The calculation of the sample size will be explained below.

3.2.2 Sample size calculation

The sample size calculation was based on a census that was conducted between September and November 2012. According to this, the household distribution was as shown in Table 3-1.

Table 3-1 Household distribution of survey census 2012

Household	Absolute no.	Percentage of total
with livestock	1351	40.4%
livestock & dogs	1711	51.17%
without	1633	48.83%
All available households	3344	100.00%

Under the assumptions of a confidence level of 90%, a precision of 7%, an estimated prevalence of trypanosomiasis in cattle of 30% and a rate of homogeneity of 0.18, the sample size was calculated using Csurvey. Trypanosomiasis was taken as a basis since it was expected to show highest prevalence based upon evidence from previous studies. Cattle showed the highest prevalence of trypanosomiasis and Table 3-2 shows the calculation based on cattle numbers. From our census we know that there are 27.41% (469/1711) cattle-keeping households in the study area. The amount of cattle clusters to be sampled at least is 38 as calculated by Csurvey. But since it was the intention to sample four other animal species as well, it was necessary to add clusters to have enough households to get a representative amount of animals of each species. Therefore a multiplication factor representing the proportion of cattle-keeping households to mammal-keeping households (1711/469) was calculated. This finally gives us the total number of clusters seen below.

Table 3-2 Sample size calculation for cluster sampling based on trypanosomiasis in animals; the rate of homogeneity is taken from trypanosomiasis in cattle in Nigeria

Species	number of animals	number of cattle keeping households	percentage from all livestock keeping households	average number of animals per HH	number of cattle clusters	multiplication factor	total number of animal clusters	level of confidence	precision	estimated prevalence	rate of homogeneity
cattle	3169	469	27.41%	6.76	38	3.64	139	90%	7%	30%	0.18

The expected numbers of animals to be sampled can be seen in Table 3-3.

Table 3-3 Number of animals per species to be sampled in above specified number of clusters

Species	Number of animals	No of mammal-keeping households	Average no of animals per household	No of clusters to be sampled	Expected no of animals to be sampled
cattle	3169	1711	1.85	139	257
goats	5679	1711	3.31	139	461
sheep	265	1711	0.15	139	22
pigs	3106	1711	1.81	139	252
dogs	2960	1711	1.73	139	240
Total					1232

It has to be noted that not all mammal-keeping households own all species and therefore the average number of animals per household is statistically calculated and not actual.

3.2.3 Sampling protocol

The calculated 139 mammal-keeping households received random numbers using an internet website (www.randomizer.org). The census households were numbered in sequence of their data entry. The random numbers obtained from the website were then applied to the census household list and in that way the households were randomly selected. In case the household had moved, was not to be found, unwilling to participate or no longer owned any animals, the next household in the list was chosen as replacement.

Within a household, all mammals were intended to be sampled, regardless of species.

3.2.4 Animal health questionnaire

The questionnaires were conducted and recorded on tablets using droidSurvey software. Three tablets used were Samsung Galaxy Tab 2 with a 7 inch screen, 3G and 8GB memory. The remaining two were of similar quality with a bigger screen size from Samsung. The advantages of using digital technology has been mentioned in many other studies and therefore it was decided to use it in this survey too (Aanensen *et al.*, 2009; Seebregts *et al.*, 2009). Each animal sampled in the household should have a matching questionnaire. The identification was based on an area code, household number, number of the animal sampled per household, species, age in months and sex of the animal. If the age of the animal was unknown by its owner, it was determined by inspection of the teeth.

Questions asked were in relation to the reproductive status of the animal, if the animal was used for traction, its body condition score, any disease signs reported or seen, any treatments conducted and if ticks were observed. A detailed overview of the questions asked can be found in the annex.

Figure 3-5 The questionnaire was recorded into a tablet by a second person while the animal was sampled

Figure 3-5 shows a colleague recording the answers onto the tablet while other colleagues are taking the blood sample.

3.2.5 Laboratory analysis

The laboratory analyses for these studies were undertaken in five different places. The African swine fever diagnosis was conducted by the international reference laboratory at the Pirbright Institute in the UK. The cysticercosis diagnostics were conducted at the University of Zambia, as were some of the brucellosis samples. Other brucellosis samples were tested in the laboratory of Msoro hospital and at the Tsetse and Trypanosomiasis station in Kakumbi. The laboratory analysis of trypanosomiasis and TBI samples was conducted at the University of Edinburgh.

3.2.5.1 Sample taken

Each sample for each disease and each questionnaire was given a unique identification (ID). It started with an area code (N for North and S for South), a household number, a sample number, species code, age of the animal in months and sex of the animal.

To prepare blood sampling, animals had to be restrained according to animal welfare rules. Cattle usually had to be restrained by lying on the floor. Sheep and goats were held between the legs of one person while a second person would take the sample. Dogs would be muzzled and held by one person. Depending on the ease of control of the animal to be sampled, this could be quite a challenging task. Pigs were also held on the floor with their mouth tied.

The blood for trypanosomiasis and tick-borne infections (TBI) was collected on FTA cards (Whatman) from all species. Peripheral blood was sampled from the ear vein of the animal by pricking with a small needle (Figure 3-6).

Figure 3-6 Blood for the FTA card was taken with the help of a micro capillary from the ear vein

The blood was then sucked up in a heparinised capillary tube of 75 µl. Ideally two tubes were needed to cover one sample space on the FTA card (125 µl). An FTA card has four circles, i.e. it has space for four samples. The identification number was written below to be able to link it to the sample (Figure 3-7).

Figure 3-7 The blood sample was transferred to a FTA card using a microcapillary tube

For brucellosis, serum was needed from all sexually mature animals except pigs. The Rose Bengal test is not ideal for testing individual pigs and was therefore not conducted in this species (FAO). The serum was taken from the jugular vein for the ruminants and from the cephalic vein for dogs using vacutainers (Figure 3-8). Similarly, the samples for cysticercosis and ASF were taken except that heparinised blood was required. All tubes received a unique animal sample ID.

Figure 3-8 For brucellosis testing blood was taken from the jugular vein (© N.Anderson)

Two more people were present during the sampling process; one was filling in the questionnaire and another one helping with labelling and storing of the FTA cards and tubes. The FTA cards were hung in the car or on a tree until the blood was dry.

3.2.5.2 Sample preparation

To prepare the samples for the subsequent diagnostic methods used, the DNA was extracted from the FTA card. To start with, five 3 mm holes were punched into the circle of the FTA card. Using fewer punched discs will lead to an underestimation of the true prevalence (Cox *et al.*, 2010). The discs were allowed to fall into a sterile Eppendorf tube (1.5 ml) which was held below the card during the process. After punching one sample, fifteen discs were punched out of clean blank paper (180 gsm) and discarded before the next sample. The intention was to reduce contamination through the punch from one sample to the next. Additionally, wash controls were punched, one at the beginning, one at the end and in-between after every twentieth sample. These wash controls contained five discs of the blank paper. They were treated like the samples and were an indicator for contamination.

After this, the discs were washed twice with FTA wash for 13 min each and then twice with TE buffer also for 13 min each. Following the washing, the discs were transferred into sterile PCR tubes. Once the discs were dry, 100 µl of a 5% chelex solution was added to the sample. The samples were then heated for 30 min at 90°C to elute the DNA.

3.2.5.3 Diagnostic techniques used for trypanosomiasis

Several PCR methods were used for the detection of trypanosomes.

3.2.5.3.1 <u>ITS PCR</u>

ITS PCR as described by Njiru *et al.* is used to detect *T. congolense (*Kilifi/Savannah/Forest*)*, *Trypanozoon*, *T. simiae (Tsavo)*, *T. vivax* and *T. godfreyi* (Njiru *et al.*, 2005). The protocol was adapted and the master mix prepared as described in the table below; 25 µl of the master mix was added to 5 µl of the sample. For positive controls, *T. b. brucei* and *T. b. rhodesiense* had been previously passaged in laboratory mice as described by Welburn and Maudlin by Dr Ewan MacLeod (Welburn and Maudlin, 1987). DNA was obtained through use of a Qiagen DNeasy kit and stored at -20°C.

Initially, the ITS master mix was prepared using Biotaq Red DNA polymerase until its production was stopped.

Table 3-4 ITS PCR master mix preparation using Biotaq Red DNA polymerase for a total of 25 µl per sample (adapted from (Njiru *et al.*, 2005))

ITS PCR reagent	Composition	Amount (in µl)
10x NH_4 reaction buffer	670 mM Tris-HCI (pH 8.8 at 25°C), 160 mM $(NH_4)_2SO_4$, 100 mM KCI, 0.1% stabilizer	2.5
$MgCl_2$	50 mM	1
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix	10 mM (ITSCF, ITSBR)	2.5
Water	DNA-se/RNA-se free MilliQ water	12.8
Biotaq Red DNA Polymerase from Bioline®	1 unit/ µl	1
DNA template		5

For the following samples, Mango Taq was used. One batch of samples was tested with the two different master mixes to ensure congruency of the results.

Table 3-5 ITS PCR master mix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Njiru *et al.*, 2005))

ITS PCR reagent	Composition	Amount (in µl)
Buffer	5x Mango Taq colored reaction buffer Bioline®	5
$MgCl_2$	50 mM	1
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix	10 mM (ITSCF, ITSBR)	2.5
Water	DNA-se/RNA-se free MilliQ water	10.8
Mango Taq	0.2 units/ µl	0.2
DNA template		5

The primers used for ITS PCR are listed in the table below.

Table 3-6 Primer sequences used for ITS PCR (Njiru *et al.*, 2005)

Primer	Sequence (from 5’-3’)
ITSCF	CCG GAA GTT CAC CGA TAT TG
ITSBR	TTG CTG CGT TCT TCA ACG AA

The ITS cycle conditions can be seen in the table below.

Table 3-7 ITS cycling conditions (adapted from (Njiru *et al.*, 2005))

ITS cycling steps	temperature	time
1	95 °C	5 min
2	94 °C	40 sec
3	58 °C	40 sec
4	72 °C	90 sec
5	Back to step 2 for 34x	
6	72 °C	2 min
7	4 °C	forever

The expected band sizes are between 250-700 bp.

3.2.5.3.2 *T. godfreyi* PCR

Another 12 samples were tested for *T. godfreyi* using a species-specific PCR developed by McNamara (McNamara *et al.*, 1994). The reason for this was that the result in the ITS PCR was not uniquely identifiable and from previous studies one would expect less samples positive for *T. godfreyi*.

The recipe for the preparation of the master mix is shown in Table 3-8.

Table 3-8 *Trypanosoma godfreyi* master mix preparation for a total of 25 µl per sample (adapted from (Masiga *et al.*, 1996))

***T. godfreyi* PCR reagent**	**Composition**	**Amount (in µl)**
Buffer	5x Mango Taq colored reaction buffer Bioline®	5
$MgCl_2$	50 mM	0.75
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix	10 mM (DGG1, DGG2)	2.5
Mango Taq	0.2 units/ µl	0.2
Water	DNA-se/RNA-se free MilliQ water	11.35
DNA template		5

The primer sequences for the *T. godfreyi* PCR is shown in Table 3-9.

Table 3-9 Primer sequences used for Trypanosoma godfreyi (McNamara *et al.*, 1994)

Primer	**Sequence (from5'-3')**
DGG1	CTG AGG CTG ACC AGC GAC TC
DGG2	CTA CGC TAT GCC AAT ACG CC

The cycle conditions for this PCR are shown in Table 3-10.

Table 3-10 *Trypanosoma godfreyi* cycling programming (adapted from (McNamara *et al.*, 1994))

***T. godfreyi* cycling steps**	**temperature**	**time**
1	94 °C	1 min
2	94 °C	30 sec
3	60 °C	1 min
4	72 °C	30 sec
5	Back to step 2 for 29x	
6	72 °C	5 min
7	4 °C	forever

The expected band size for *T. godfreyi* is at 149 bp.

3.2.5.3.3 Additional PCR protocols used

For further characterisation of *T. brucei s.l.* species the multiplex PCR was used (Picozzi *et al.*, 2008). In certain cases it was needed to confirm the animal species, because the ID was not legible or faulty. For this, cytochrome B PCR was used followed by sequencing. The chapter on human health gives further details of this process.

3.2.5.3.4 Presentation of the results

In the classical PCR, the results are presented on an agarose gel. For this the gel was prepared and placed into gel tanks filled with buffer covering the gel. The samples were loaded into small wells in the gel in a precise order. A DNA ladder was added in one of the wells to be able to quantify the base pair level the sample has reached. The gel was then exposed to an electric current for 45-90 min depending on the expected length of the target.

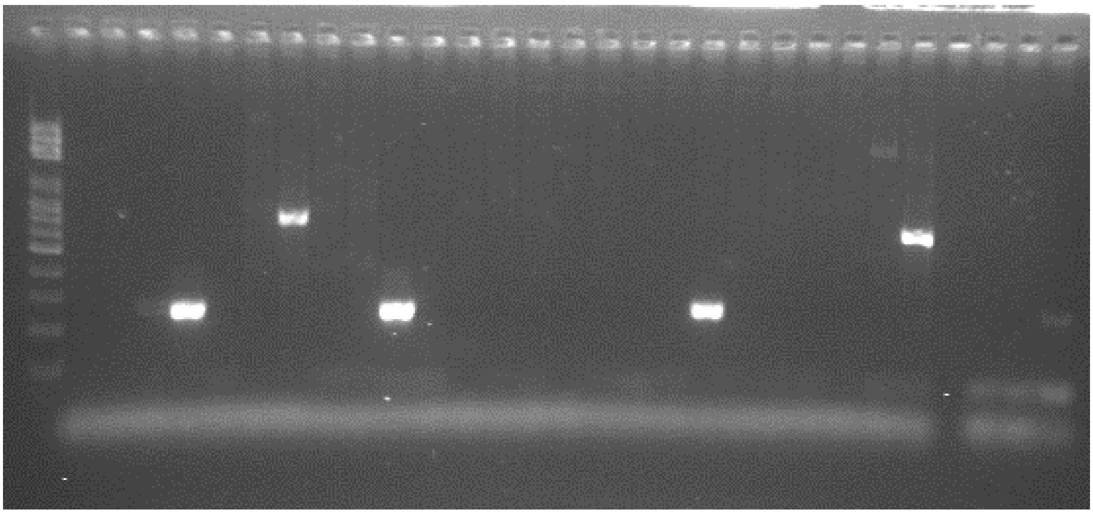


Figure 3-9 A typical image of an agarose gel with results from an ITS PCR

Figure 3-9 shows how the results for the different parasite species look like on a gel.

3.2.5.4 Diagnostic techniques used for tick-borne diseases

The diagnostic test used for tick-borne diseases in this work is called reverse line blot (RLB). It has been established and validated at the University of Edinburgh by a colleague (Lorusso, 2014). The assay is able to detect 37 microorganisms at the same time and is therefore a very time and cost-effective tool to use.

It has to be noted that the species description *Ehrlichia sp. Omatjenne* is used, but newer publications assign this species to a different genus, namely *Anaplasma*. Zweygarth et al mention in their publication that the sequence of *Anaplasma sp. Omatjenne* is identical to *Ehrlichia sp. Omatjenne* and 99.5% identical to *Anaplasma (Ehrlichia) platys* (Zweygarth *et al.*, 2006). To reduce confusion, *Ehrlichia sp. Omatjenne* will be used throughout this thesis.

3.2.5.4.1 <u>RLB PCR</u>

For the RLB, each sample had to be amplified by three PCRs, one targeting a 460-540 bp long fragment from the 18S ribosomal RNA (rRNA) gene for *Theileria* and *Babesia* spp. (Georges *et al.*, 2001), a second one targeting a 460–520 bp long fragment from the V1 hypervariable region of the 16S SSU rRNA gene for *Ehrlichia* and *Anaplasma* spp. (Bekker *et al.*, 2002) and a third one with 350–400 bp in the 16S ribosomal RNA gene for *Rickettsia* spp. (Christova *et al.*, 2003).

A preparation of the master mix can be found in Table 3-11. It is the same for all three PCRs except for the primers added.

Positive controls included 2.5µl of DNA from Theileria parva (ACCESSION No. KJ095110), Ehrlichia canis (ACCESSION No. KJ095115), and rickettsial DNA >98% similar to Rickettsia africae (AN: JX101606) (Alberdi et al., 2012), for the three aforementioned PCRs (Lorusso, 2014).

Table 3-11 RLB PCR mastermix preparation for a total of 25 µl per sample (Lorusso, 2014)

RLB PCR reagent	Composition	Amount (in µl)
5x Phire Reaction Buffer	160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCI (pH 8.8 at 25°C) and stabilizers	5
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Fwd primer	20 pmol/ µl (Ehr-F/RLB-F2/Rick-F) in MilliQ water	0.5
Rev primer	20 pmol/ µl (Ehr-R/RLB-R2/Rick-R) in MilliQ water	0.5
Phire Hot Start II Polymerase Taq	2 units/ µl	0.125
Water	DNA-se/RNA-se free MilliQ water	15.875
DNA template		2.5

Table 3-12 shows the sequences of the primers used for the three PCRs. It is important to note that the reverse primers here are labelled with biotin. This is essential for the subsequent blotting.

Table 3-12 Primers used for the RLB protocol (Lorusso, 2014)

Primer	Sequence (5' – 3')	Orientation	Tm* (ºC)	Reference
Ehr-F (a.k.a. 16S8FE)	GGA ATT CAG AGT TGG ATC (A/C)TG G(C/T)T CAG	+	61	(Schouls *et al.*, 1999)
Ehr-R (aka BGA1B-new)	Biotin–CGG GAT CCC GAG TTT GCC GGG ACT T(C/T)T TCT	-	69.5	(Bekker *et al.*, 2002)
RLB-F2	GAC ACA GGG AGG TAG TGA CAA G	+	57.9	(Georges *et al.*, 2001)
RLB-R2	Biotin-CTA AGA ATT TCA CCT CTG ACA GT	-	53.7	
Rick-F	GAA CGC TAT CGG TAT GCT TAA CAC A	+	66.9	(Christova *et al.*, 2003)

Rick-R	Biotin-CAT CAC TCA CTC GGT ATT GCT GGA	-	69.4	

The conditions for amplification of DNA are given in Table 3-13.

Table 3-13 RLB PCR cycling programming (Lorusso, 2014)

RLB cycling steps	temperature	time
1	98 °C	30 sec
2	98 °C	5 sec
3	67 °C decrease by 1 °C every cycle	5 sec
4	72 °C	7 sec
5	Cycle to step 2 for 9x	
6	98 °C	5 sec
7	57 °C	5 sec
8	72 °C	7 sec
9	Cycle to step 6 for 39x	
10	72 °C	1 min
11	4 °C	forever

When the samples were removed from the machine the positive and negative controls were put on an agarose gel to confirm that the PCR had been working and that there was no contamination until that point. It was not necessary to do so for all samples.

3.2.5.4.2 Reverse line blot

The method is well described with illustrations in other sources (Kong and Gilbert, 2006; Lorusso, 2014; O'Sullivan *et al.*, 2011) but, in brief it comprises the following stages. On completion of the PCR, the three PCR products were mixed together with buffer for each sample and were then heated to denature the DNA and in the next step placed on the activated membrane in the Miniblotter with each sample and control filling one column. The rows were loaded with probes from the microorganisms chosen to be detected. The sequences of the probes can be found in the Table 3-14.

Table 3-14 Sequences of probes that were used on the RLB membrane (adapted from (Lorusso, 2014))

	Tick-borne microorganism genera/ species	**Probe sequence (from 5'-3')**	**Reference**
1	Ehrlichia/ Anaplasma catch all	GGGGGAAAGATTTATCGCTA	(Bekker *et al.*, 2002)
2	Anaplasma bovis	GTAGCTTGCTATG(A/G)GAACA	(Georges *et al.*, 2001)
3	Anaplasma centrale	TCGAACGGACCATACGC	(Bekker *et al.*, 2002)
4	Anaplasma marginale	GACCGTATACGCAGCTTG	(Bekker *et al.*, 2002)
5	Anaplasma phagocytophilum	TTGCTATAAAGAATAATTAGTGG	(Schouls *et al.*, 1999)
6	Anaplasma phagocytophilum	TTGCTATGAAGAATAATTAGTGG	(Schouls *et al.*, 1999)
7	Anaplasma phagocytophilum	TTGCTATAAAGAATAGTTAGTGG	(Schouls *et al.*, 1999)
8	Anaplasma phagocytophilum	TTGCTATAGAGAATAGTTAGTGG	(Schouls *et al.*, 1999)
9	Anaplasma platys	GTCGTAGCTTGCTATGATA	unpublished
10	Ehrlichia ruminantium	AGTATCTGTTAGTGGCAG	(Bekker *et al.*, 2002)
11	Ehrlichia ruminantium new	ATTTCTAATAGCTATTCCAT	(Allsopp *et al.*, 1999)
12	Ehrlichia sp. Omatjenne	CGGATTTTTATCATAGCTTGC	(Bekker *et al.*, 2002)
13	Ehrlichia chaffeensis	ACCTTTTGGTTATAAATAATTGTTA	(Schouls *et al.*, 1999)

14	Theileria/ Babesia catch all	TAATGGTTAATAGGA(A/G)C(A/G)GTTG	(Gubbels *et al.*, 1999)
15	Babesia catch all 1	ATTAGAGTGTTTCAAGCAGAC	Nijhof (unpublished)
16	Babesia catch all 2	ACTAGAGTGTTTCAAACAGGC	Nijhof (unpublished)
17	Babesia bigemina	CGTTTTTTCCCTTTTGTTGG	(Gubbels *et al.*, 1999)
18	Babesia bovis	CAGGTTTCGCCTGTATAATTGAG	(Gubbels *et al.*, 1999)
19	Babesia caballi	GTGTTTATCGCAGACTTTTGT	(Oosthuizen *et al.*, 2009)
20	Babesia caballi 3	GTT GCG TTK TTC TTG CTT TT	(Govender *et al.*, 2011a)
21	Babesia divergens	ACT(A/G)ATGTCGAGATTGCAC	(Oosthuizen *et al.*, 2009)
22	Theileria catch all	ATTAGAGTGCTCAAAGCAGGC	(Oosthuizen *et al.*, 2009)
23	Theileria annulata	CCTCTGGGGTCTGTGCA	(Georges *et al.*, 2001)
24	Theileria buffeli	GGCTTATTTCGG(A/T)TTGATTTT	(Gubbels *et al.*, 1999)
25	Theileria equi	TTCGTTGACTGC(C/T)TTGG	(Oosthuizen *et al.*, 2009)
26	Theileria equi-like	TTCGTTGTGGCTTAGTTGGG	unpublished
27	Theileria mutans	CTTGCGTCTCCGAATGTT	(Gubbels *et al.*, 1999)
28	Theileria parva	GGACGGAGTTCGCTTTG	(Nijhof *et al.*, 2003)
29	Theileria taurotragi	TCTTGGCACGTGGCTTTT	(Gubbels *et al.*, 1999)
30	Theileria velifera	CCTATTCTCCTTTACGAGT	(Gubbels *et al.*, 1999)
31	Theileria sp. MSD4	GCTTATTTCGGCGACCTC	unpublished
32	Theileria sp. (duiker)	CATTTTGGTTATTGCATTGTGG	(Nijhof *et al.*, 2005)
33	Rickettsia catch all	TTTAGAAATAAAAGCTAATACCG	(Christova *et al.*, 2003)

34	Rickettsia conorii	CTTGCTCCAGTTAGTTAGT	(Christova *et al.*, 2003)
35	Rickettsia helvetica	GCTAATACCATATATTCTCTATG	(Christova *et al.*, 2003)
36	Rickettsia massiliae	TGGGGCTTGCTCTAATTAGT	(Christova *et al.*, 2003)
37	Rickettsia sp. (DnS14)/ raoulti	CTAATACCGCATATTCTCTACG	(Nijhof *et al.*, 2007)
38	Babesia canis canis	TGC GTT GAC CGT TTG AC	(Matjila *et al.*, 2008)
39	Babesia canis canis 2	TGG TTG GTT ATT TCG TTT TCG	(Matjila *et al.*, 2008)
40	Babesia canis vogeli	AGC GTG TTC GAG TTT GCC	(Matjila *et al.*, 2008)
41	Babesia rossi	CGG TTT GTT GCC TTT GTG	(Matjila *et al.*, 2008)
42	Babesia major	TCCGACTTTGGTTGGTGT	(Georges *et al.*, 2001)
43	Ehrlichia canis	TCT GGC TAT AGG AAA TTG TTA	(Matjila *et al.*, 2008)

The incubation period of the samples takes one hour. During this time, if there is any matching DNA in the sample, it will bind to the DNA of the probe. Afterwards, the residual sample was washed off the membrane before the membrane was incubated with streptavidine-peroxidase (POD) for 30 min. The streptavidine-POD binds to the biotin end of the DNA that has bound to the probes. Following this the membrane underwent several washing steps and then incubation with ECL1 and ECL2 for a few minutes. ECL1 and 2 are blotting reagents that will react with the POD and thus emit a light. Then the membrane was placed into an x-ray cassette. In the darkroom, an x-ray film was exposed to the emitting light signals of the membrane for 15 minutes and then developed like a normal x-ray picture. The result can be seen in Figure 3-39.

3.2.5.4.3 Preparing the membrane for re-use (stripping)

To be able to perform the same procedure up to 25 times with one membrane, the samples, streptavidine and blotting reagents have to be removed without removing the probes. This is done through several washing steps with different buffers and at different temperatures. The membrane was then stored in a plastic bag filled with EDTA buffer to prevent any bacterial or fungal growth. The whole process of PCR, RLB and stripping the membrane takes two days.

3.2.5.4.4 Sequencing

There was a considerable number of samples that did not show up for a specific infection, but just for the catch alls; 132 samples showed up for *Anaplasma/Ehrlichia* catch all, ten for *Theileria/Babesia* catch all, two for *Rickettsia* catch all and 17 for *Babesia* catch all. Catch all are probes with a sequence that identify samples on genus level. Due to time and budget constraints it was decided that only 10% of these unidentified samples would be sent for sequencing. Therefore following amounts of samples were sent for identification: 13/132 samples for *Anaplasma/Ehrlichia*, 1/10 sample for *Theileria/Babesia,* both *Rickettsia* catch all samples and 2/17 for *Babesia*.

3.2.5.5 Diagnostic techniques used for brucellosis

The chosen method for the diagnosis of brucellosis was the Rose Bengal test (RBT). It was conducted in laboratories close to the field sites so that environmental influences would be reduced. These were the laboratory at Msoro hospital, the Tsetse and Trypanosomiasis Research station in Kakumbi and at UNZA in Lusaka. At the end of the survey, the antigen provided by the University of Navarra, Spain, Department of Microbiology and Parasitology, was used up and new antigen had to be ordered. By the time it arrived the survey was finished and therefore the remaining samples were tested at UNZA.

Plain vacutainers with full blood were used to store the blood taken from the animal. It was directly placed in a cooling box with ice to enhance agglutination. In the laboratory, the serum was separated and stored in two separate tubes, one plain and the other one mixed with sodium azide to improve preservation. Both tubes were labelled with the original ID of the animal and stored in the field in a fridge and later in a freezer.

For the test a white tile was placed on the laboratory bench. Several spots with each 25 µl of antigen were placed on the tile. Following that, serum was added to each spot of antigen, 25 µl for cattle and dogs and 75 µl for small ruminants. The larger volume in small ruminants increases the sensitivity of the test. Then the timer was set to 4 min to read off any agglutination. Saline solution was added for the negative control and a positive control using positive serum that was delivered together with the antigen was done once a day to ensure that the antigen was still working. For the test to function properly, it is essential that antigen and serum have the same temperature that means they need to be removed from the fridge at the same time.

The antigen used was a strain of *Br. melitensis*. The diagnosis is based on the detection of the S-LPS and this is more stable for *Br. melitensis*. Since other S (smooth) *Brucellae* show cross-reactivity, the test can be applied to diagnose all S *Brucellae*. Differentiation has to be done by bacteriology (Ducrotoy *et al.*, 2015; Ducrotoy *et al.*, 2016).

3.2.5.6 Diagnostic techniques used for cysticercosis

Diagnosis of cysticercosis was conducted at the veterinary laboratory at the University of Zambia in Lusaka. They used a monoclonal antibody-based sandwich ELISA for the detection of *Taenia saginata*, *Taenia ovis* and *Taenia solium* antigen in serum (Brandt *et al.*, 1992; Dorny *et al.*, 2003). The validity of the test was described by Dorny et al. (Dorny *et al.*, 2004).

3.2.5.7 Diagnostic techniques used for African swine fever

The samples tested for African swine fever were sent to the Pirbright Institute in the United Kingdom. They are a world reference laboratory accredited by OIE for African swine fever. They used RT-PCR to diagnose ASF presence.

3.3 Results

The demographic data presented here are based on three different data sets; FTA card samples, brucellosis samples and animal health questionnaire answers. All use the same identification number per sample and can therefore be matched with each other. However, sometimes certain entries were missing and were not available to match with another entry. Hence, analysis relating to more than one data set can only be conducted when all those data are available from the required data sets. This means that if for example a hypothesis needs information from the FTA samples as well as from the questionnaire, only entries that exist in both data sets were used. The aim was to have an FTA sample and a questionnaire from each animal. Due to errors, animals running away or other difficulties, not all data were available for analysis, thus minor differences exist between the data sets.

The software used for the analysis of animal health related data is mentioned in the introductory chapter.

The animal health survey was conducted between April and August 2013. This is dry and cool season in the study area and one would expect fewer infections due to decreased vector populations (tsetse flies and ticks).

There were several problems encountered when sampling animals in the households. First of all, in this area it is uncommon to restrain animals, be it individually or in a kraal/ stable as a group or herd. Therefore it was always a challenge to catch and restrain the animal to be sampled in a safe way for the animal and the field worker. It was also essential to stick to animal welfare regulations. Many dogs were not used to close contact with humans, let alone being touched. Our work interfered often with the usual human-animal relationships in this area, animals became suspicious and ran away or did not come back before us leaving the area.

Another quite common problem was the refusal of owners to have their animals sampled. This could be for the whole household as it could be only for one species and not the other one. Overall, it was our aim to sample all animals living in a household, but we were not successful in all households.

Some households keep animals in custody or have given their animals in custody. For the study of animal health, all animals living at one geographical place were considered a unit. The discrepancy of animal ownership was taken into consideration in the household questionnaire.

3.3.1 Questionnaire related information

The following sections give an overview of the information received from the animal health questionnaires (AHQ). For each animal, one animal health questionnaire should be available. Figure 3-10 illustrates the species distribution of the AHQ.

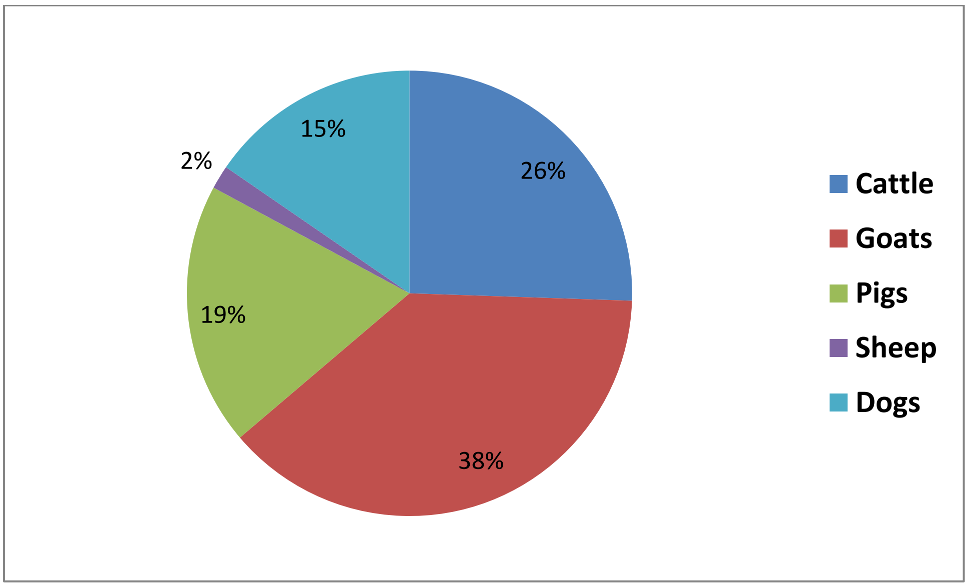


Figure 3-10 Distribution of animal health questionnaires conducted (n=1226)

3.3.1.1 Goats

There were 64 households that answered questions about their goats. The average herd size was 7.3 with a range of 1-22 animals. The distribution for age and sex can be found in Table 3-15.

Table 3-15 Goats life stage demography based on AHQ (entire means confirmed non-castrated)

age categories	**reproductive status**	**female**	**female**	**male**	**male**
<4 months	juvenile	47	13.6%	38	30.9%
5-12 months	with suckling	7	2.0%	-	-
	pregnant	12	3.5%	-	-
	none of the above	66	19.1%	-	-
	unknown	1	0.3%	-	-
>12 months	with suckling	50	14.5%	-	-
	pregnant	88	25.5%	-	-
	none of the above	71	20.6%	-	-
	unknown	3	0.9%	-	-
5-12 months	unknown	-	-	57	46.3%
	entire	-	-	2	1.6%
>12 months	unknown	-	-	25	20.3%
	entire	-	-	1	0.8%
Total		345	100%	123	100%

The body condition score can often show the nutritional status of an animal and thus its resilience to diseases. Well nourished animals are less likely to be ill or acquire an infection. The observed body condition score of goats in this study is displayed in Figure 3-11.

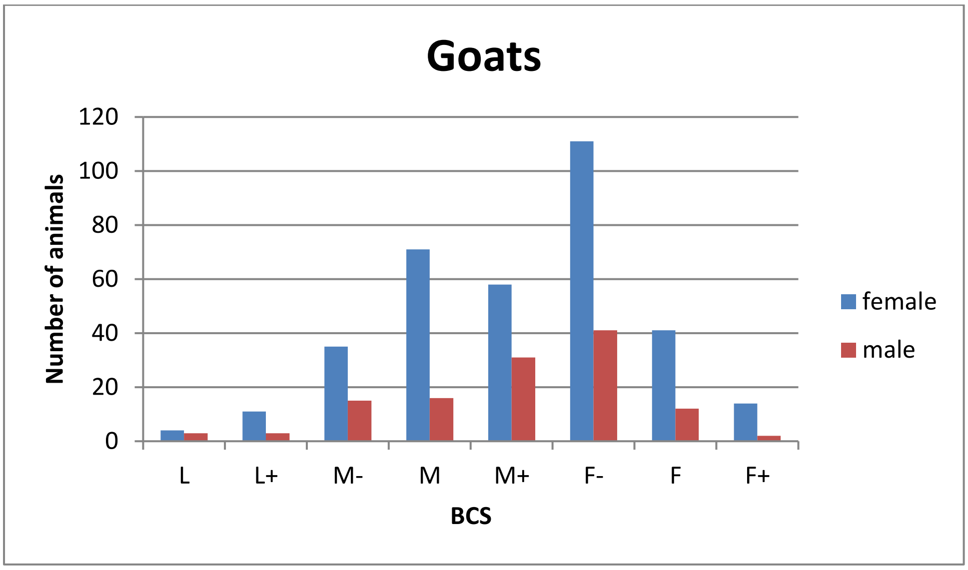


Figure 3-11 Body condition score of goats sampled (L=lean, M=medium, F=fat)

At the time of sampling, 29% (100/345) of the female animals were pregnant (age 7-120 months) and 16.5% (57/345) of them had suckling kids. 4.8% (16/334) of female animals were reported to have had abortions or stillbirths, with eleven animals who had one, one animal who had two, three animals who had three and one animal with four dead offspring. Contrary to that, 51% (171/336) of female animals gave birth to living ones with the majority of 80% (137/171) giving birth to 1-4 kids, 12.3% (21/171) giving birth to 5-8 kids and 7.6% (13/171) giving birth to 9-21 kids. The age of first birth was between 5-41 months. None of the male goats were castrated.

Clinical signs were rarely seen in small ruminants. None of the animals was treated and none of them had ticks attached. But bad claws as shown in the Figure 3-12 were seen regularly in the study area. This was mainly related to mismanagement of housing the animals. If the stables were built in such a way that the animals do not stand in their faeces and have an opportunity to climb a bit as can be the case in an elevated stable on pillars, there was less damage to claws.

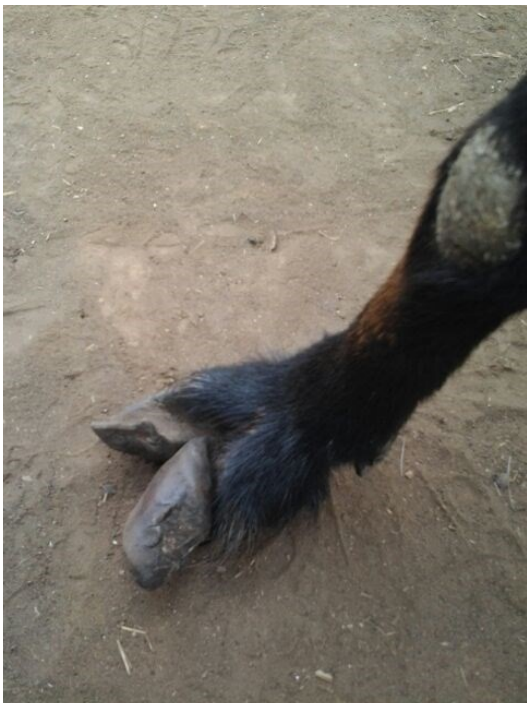


Figure 3-12 Bad claw management in a goat (picture taken from animal health questionnaire)

3.3.1.2 Pigs

In 45 households farmers were asked questions about their pigs. The average herd size was 5.2 with a range of 1-18 animals. The distribution for age and sex can be found in Table 3-16.

Table 3-16 Pig life stage demography based on AHQ

age categories	**reproductive status**	**female**	**female**	**male**	**male**
<4 months	juvenile	40	25.2%	37	48.7%
5-7 months	pregnant	3	1.9%	-	-
	none of the above	20	12.6%	-	-
	unknown	3	1.9%	-	-
>7 months	with suckling	18	11.3%		
	pregnant	41	25.8%		
	none of the above	30	18.9%		
	unknown	4	2.5%		
5-7 months	unknown	-	-	14	18.4%
	entire	-	-	2	2.6%
>7 months	unknown	-	-	22	28.9%
	entire	-	-	1	1.3%
Total		159	100%	76	100%

The observed body condition score of pigs in this study is displayed in Figure 3-13.

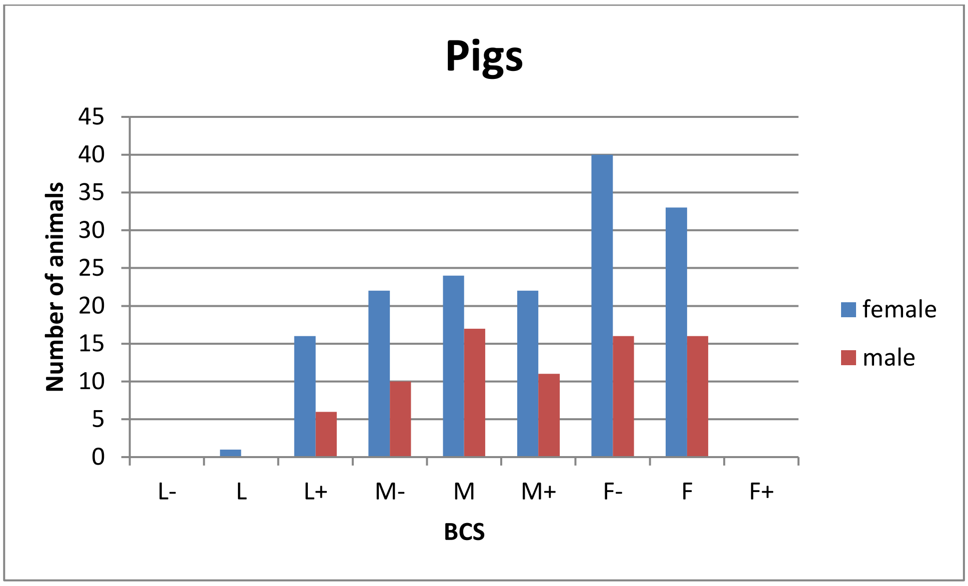


Figure 3-13 Body condition score pigs

During the survey, 28% (44/158) of female pigs were pregnant (age 6-36 months) and 11.4% (18/158) of female pigs (age 10-48 months) had suckling piglets. 20.5% (30/146) of female had already living offspring, 30% (9/30) of those had 1-4 offspring, 50% (15/30) had 5-8 offspring and 20% (6/30) had 12-24 living offspring. The age of first birth was between 8-36 months. Nearly 3 % (4/146) had already abortions or stillbirths with one pig one, two pigs two and one pig four dead offspring. One female pig aged 24 months was neutered. It would have been interesting to know who and how they did it, since there are no veterinary services conduction pig castrations in the area.

Lice as seen in Figure 3-14 were seen very often on pigs. It was not sure if owners realized that these were ecto-parasites that could impair the health status of their animals. While the survey was conducted, 3.4% (8/235) of pigs had ticks attached to their body.

Figure 3-14 A pig with white lice (picture taken from animal health questionnaire)

3.3.1.3 Dogs

In 68 households people answered questions about their dogs. The average pack size was 2.8 with a range of 1-8 animals. The distribution for age and sex can be found in Table 3-17.

Table 3-17 Dog life stage demography based on AHQ

age categories	reproductive status	female	female	male	male
<2 months	juvenile	24	24.5%	22	24.2%
3-7 months	with suckling	2	2.0%	-	-
	none of the above	19	19.4%	-	-
8-14 months	with suckling	2	2.0%	-	-
	pregnant	3	3.1%	-	-
	none of the above	7	7.1%	-	-
	unknown	1	1.0%	-	-
>14 months	with suckling	8	8.2%	-	-
	pregnant	6	6.1%	-	-
	none of the above	25	25.5%	-	-
	unknown	1	1.0%	-	-
3-7 months	unknown	-	-	13	14.3%
8-14 months	unknown	-	-	14	15.4%
	entire	-	-	3	3.3%
>14 months	unknown	-	-	34	37.4%
	entire	-	-	5	5.5%
Total		98	100%	91	100%

During blood sampling, 9.4% (9/96) of bitches were pregnant (age 12-24) and 10.4% (10/96) of bitches (age 12-48) had suckling puppies. 21.3% (20/94) have had living offspring with 11 bitches 1-3 puppies, 8 bitches 4-6 puppies and one bitch 12 puppies. The age of first birth was between 8-33 months. Five dogs (5.3%, 5/94) had one abortion or stillbirth each.

None of the dogs were neutered or castrated, but a dog not participating in the survey was seen in a lot of pain with an open scrotum after a failed attempt to castrate it. Another one was seen with a penis prolapse. It was caught in order to reposition the penis.

The observed body condition score of dogs in this study is displayed in Figure 3-15.

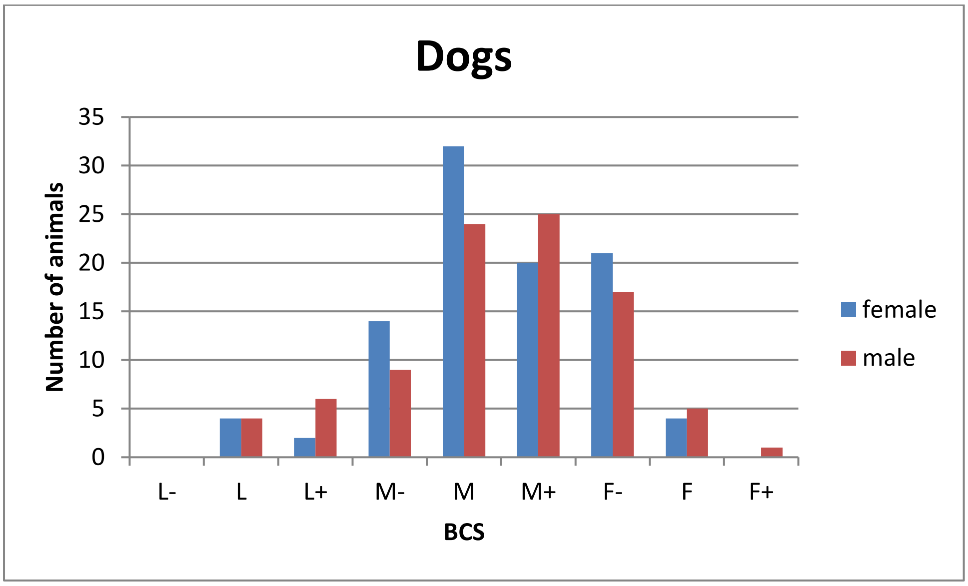


Figure 3-15 Body condition score dogs

Many dogs were observed with mange and bloated tummy, probably due to worms, as seen in Figure 3-16. 9% (17/189) of dogs were found with ticks attached to their body. One puppy had a completely white skin due to its anaemic state and the fleas attached to its body were motionless sucking the last bit of blood out of its body. The puppy did not show any liveliness and was allowed to be relieved of its agony. Two dogs were reported to be treated with a rabies vaccine.

Figure 3-16 A puppy with mange and emaciation (picture taken from animal health questionnaire)

Another common problem seen in dogs especially in puppies was myasis as shown in Figure 3-17. Where found, the larvae were removed.

Figure 3-17 Myasis on the chin of a puppy (picture from animal health questionnaire)

Another well known disease in the area affecting the reproduction of dogs was the transmissible venereal tumour (Figure 3-18). It is transmitted through the mating process and treatment is very difficult and costly, because it is done through chemotherapy or radiotherapy. Most dogs with this disease will be killed by their owners (personal communication F. Hangandu).

Figure 3-18 Transmissible venereal tumour in a bitch (picture from animal health questionnaire)

In general, dogs did not look healthy, but it was difficult to identify the cause.

3.3.1.4 Cattle

Farmers from 38 households were asked questions about their cattle. The average herd size was 8.2 with a range of 1-41 animals. The distribution for age and sex can be found in Table 3-18.

Table 3-18 Cattle life stage demography based on AHQ

age categories	**reproductive status**	**female**	**female**	**male**	**male**
<12 months	juvenile	29	19.7%	25	15.1%
12-48 months	with suckling	9	6.1%	-	-
	pregnant	10	6.8%	-	-
	none of the above	26	17.7%	-	-
	unknown	1	0.7%	-	-
>48 months	with suckling	24	16.3%		
	pregnant	18	12.2%		
	none of the above	29	19.7%		
	unknown	1	0.7%		
12-48 months	castrated	-	-	1	0.6%
	entire	-	-	1	0.6%
	unknown	-	-	62	37.3%
>48 months	castrated	-	-	7	4.2%
	entire	-	-	1	0.6%
	unknown	-	-	69	41.6%
Total		147	100%	166	100%

The reproductive information found on female adult cattle shows that 36% (43/119) of cows aged 16-120 months have never given birth to living offspring. 48% (57/119) of cows aged 12-180 months have given birth to 1-3 living calves. 10% (12/119) cows aged 60-184 months have given birth to 4-6 living calves and 7-10 calves were born by 6% (7/119) of cows aged 120-240 months. The age of first giving birth for cows was stated at 14-120 months. Furthermore, 5.6% of cows had 1-4 abortions or stillbirths.

There were only 8 oxen (castrated bulls) in the study aged 48-156 months.

The observed body condition score of cattle in this study is displayed in Figure 3-19.

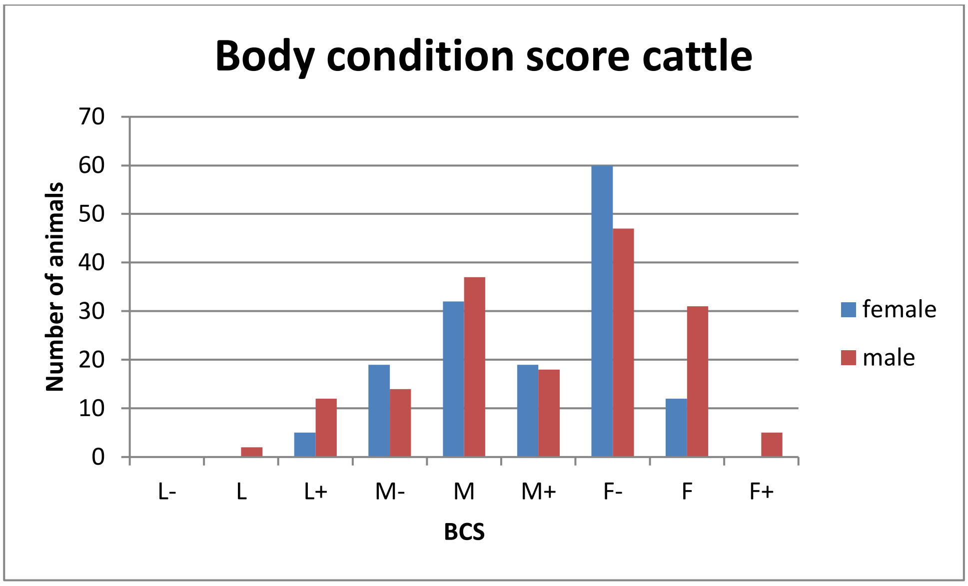


Figure 3-19 Body condition score cattle

Only 5% (16/313) of cattle showed disease signs at the time when sampling was done. Some of these signs can be seen in Figure 3-20. One shows a downer cow that was suspected to have eaten a poisonous plant.

Figure 3-20 Downer cow (picture from animal health questionnaire)

Another sign often seen were skin conditions such as that pictured in Figure 3-21 (here possibly streptothricosis caused by *Dermatophilus*). There is no topical treatment available in the area that could help with this condition. Lumpy skin disease has also a similar picture, but nothing is known about this disease in the area.

Figure 3-21 Skin lesions in a cow (picture from animal health questionnaire)

In some cases there were bigger lumps across the body or a skin rash (possibly demodicosis) (

Figure 3-22).

Figure 3-22 Skin rush in a cow (picture from animal health questionnaire)

One cow had a broken leg that the farmer attempted to treat (Figure 3-23). It is evident, not only in cattle, that veterinary support on request is desperately needed in this area.

Figure 3-23 Fracture in a cow (picture from animal health questionnaire)

Stunted growth in young cattle (Figure 3-24) was also seen quite regularly. This can have several reasons, e.g. parasites or malnutrition.

Figure 3-24 Stunted growth (picture from animal health questionnaire)

Education on how to avoid these conditions and signs seen in the pictures above could improve the situation.

3.3.1.5 Sheep

Only three households kept sheep with an average herd size of 7 and a range of 1-15 animals. The distribution for age and sex can be found in Table 3-19.

Table 3-19 Sheep life stage demography based on AHQ

age categories	reproductive status	female	female	male	male
<4 months	juvenile	1	7.7%	1	12.5%
5-12 months	unknown	1	7.7%	-	-
>12 months	with suckling	3	23.1%	-	-
	pregnant	3	23.1%	-	-
	none of the above	4	30.8%	-	-
	unknown	1	7.7%	-	-
5-12 months	unknown	-	-	2	25.0%
>12 months	unknown	-	-	5	62.5%
Total		13	100%	8	100%

The observed body condition score of sheep in this study is displayed in Figure 3-25.

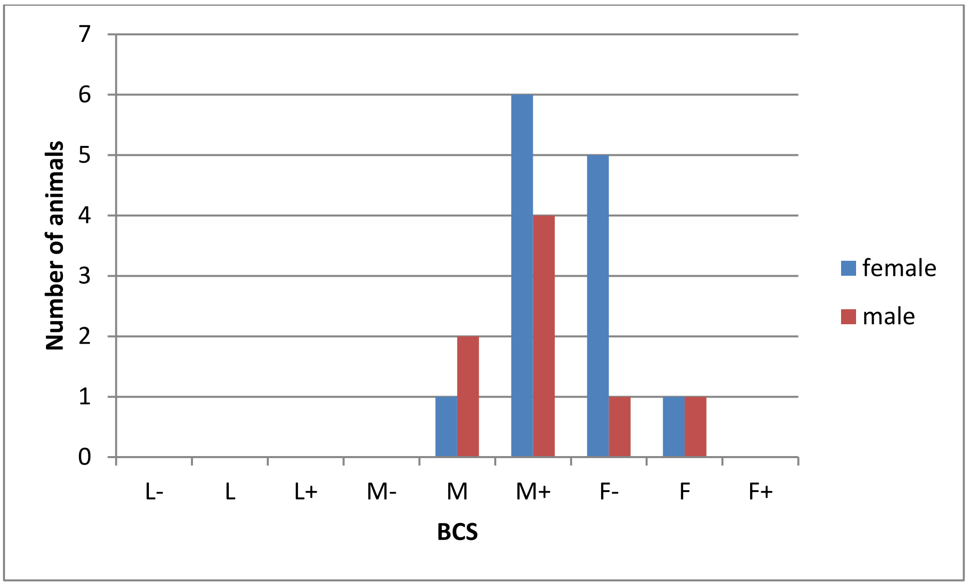


Figure 3-25 Body condition score sheep

Of the few sheep sampled, three were pregnant (age 36-54 months) and three (age 36-48) had suckling lambs. The age of first birth was between 6-36 months. There were no abortions or stillbirths mentioned. There were four animals that had 1-2 living ones born and one animal that had six living ones born.

None of the sheep had been treated. 4.8% (1/21) of the sheep had ticks on their body. 9.5% (2/21) showed clinical signs at the time of sampling. Figure 3-26 shows a sheep with an eye problem.

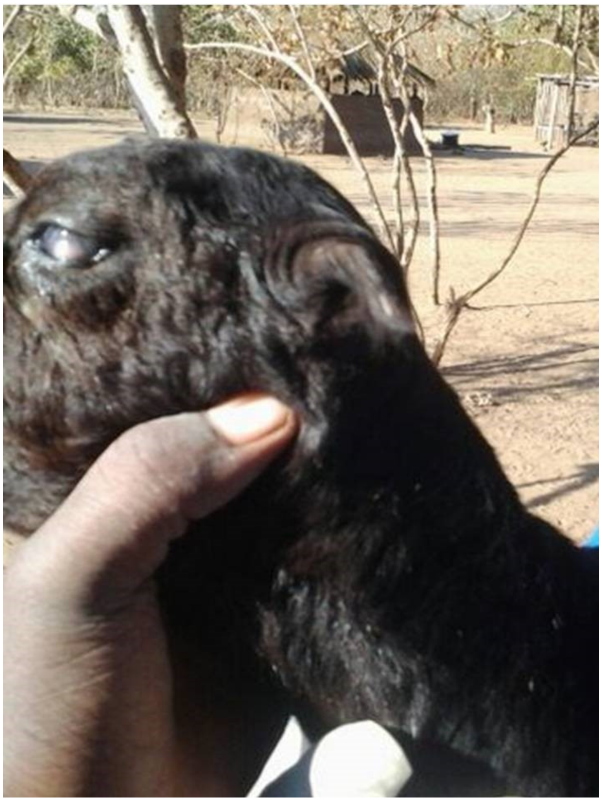


Figure 3-26 Eye problem in a sheep (picture taken from animal health questionnaire)

To summarise, it is difficult to judge the health situation in sheep since there were only few samples available.

3.3.2 Trypanosomiasis results

Trypanosomiasis was tested in all animal species sampled. An overview of the distribution is shown in Figure 3-27.

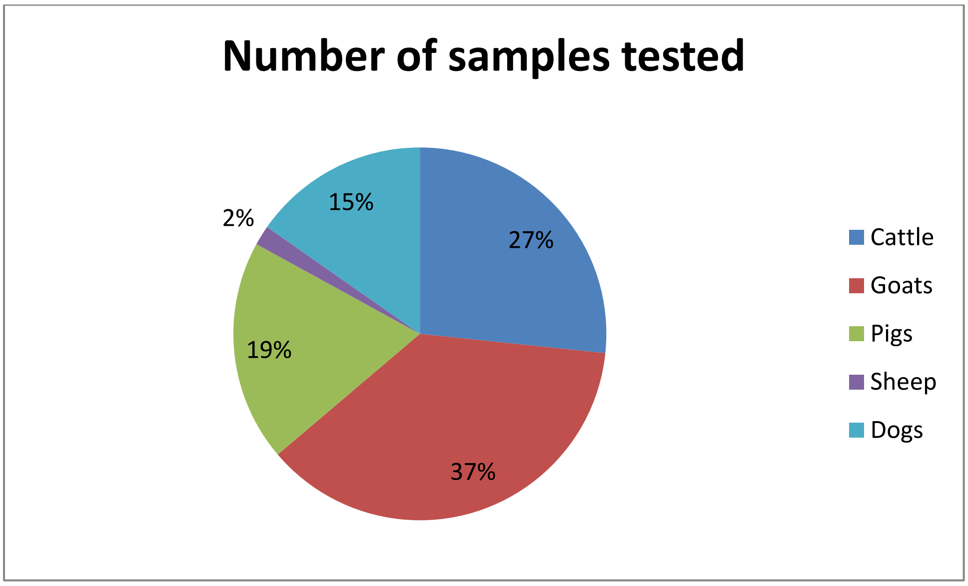


Figure 3-27 Number of samples tested for trypanosomiasis (n=1275)

Altogether 12 samples were tested using the species-specific *T. godfreyi* PCR. Looking at the ITS results, it was not always easy to distinguish *T. godfreyi* from the other species. Out of the 12 samples tested, 11 showed up positive. These results are incorporated into the animal-specific prevalence results below.

Unfortunately, the multiplex PCR (Picozzi *et al.*, 2008) described in the human health chapter did not generate any usable results despite several efforts and amendments to the protocol. Therefore no conclusion can be made about which subspecies of *Trypanozoon* was found in the processed samples.

3.3.2.1 Trypanosomiasis in cattle

Cattle from 35 households were sampled for trypanosomiasis and tick-borne infections. The age and sex distribution of these samples is shown in a pyramid graph (Figure 3-28).

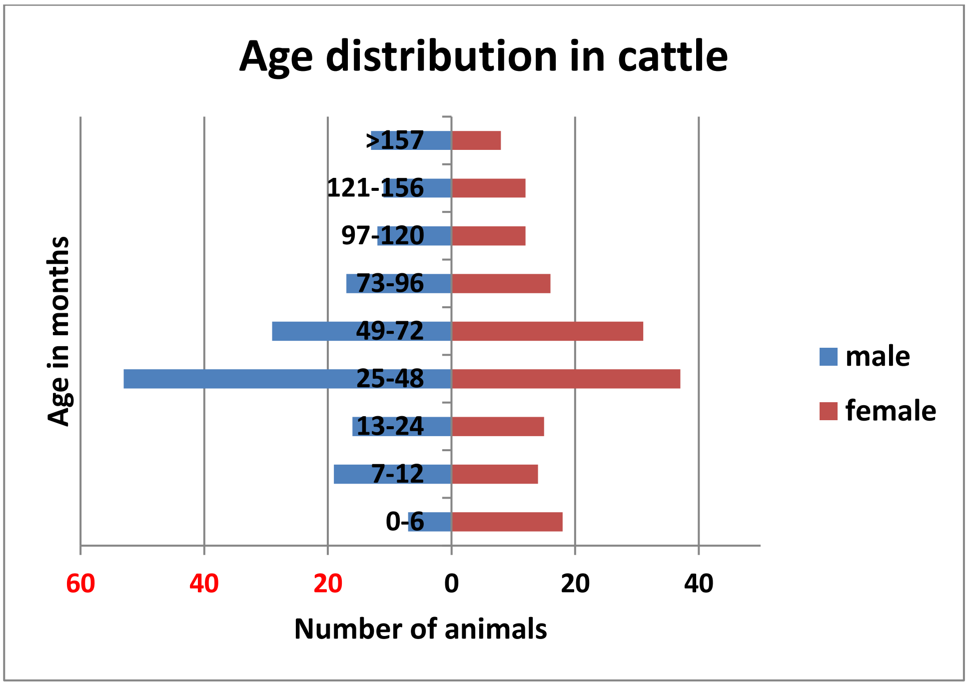


Figure 3-28 Cattle population sampled (n=340)

Altogether 340 blood samples were tested and resulted in 22.4% (76/340) of samples showing positive results for trypanosomes. There were 90.8% (69/76) single infections and 9.2% (7/76) double trypanosome infections looking at all infections. The prevalence is as follows for each parasite: *T. congolense* 5.9% (95% CI 2-9.8, DE 2.2), *T. congolense* Kilifi 1.5% (95% CI -0.008-2.9, DE 1.2), *T. vivax* 15% (95% CI 10.6-19.4, DE 1.3), *Trypanozoon* 1.8% (95% CI 0.4-3.2, DE 0.9) and *T. godfreyi* 0.3% (95% CI -0.3-0.9, DE 1).

In Figure 3-29, one can see the distribution of households with positive cattle as compared to all households keeping cattle. It is clearly visible that despite the relatively low number of households keeping cattle; nearly all farmers have infected cattle.

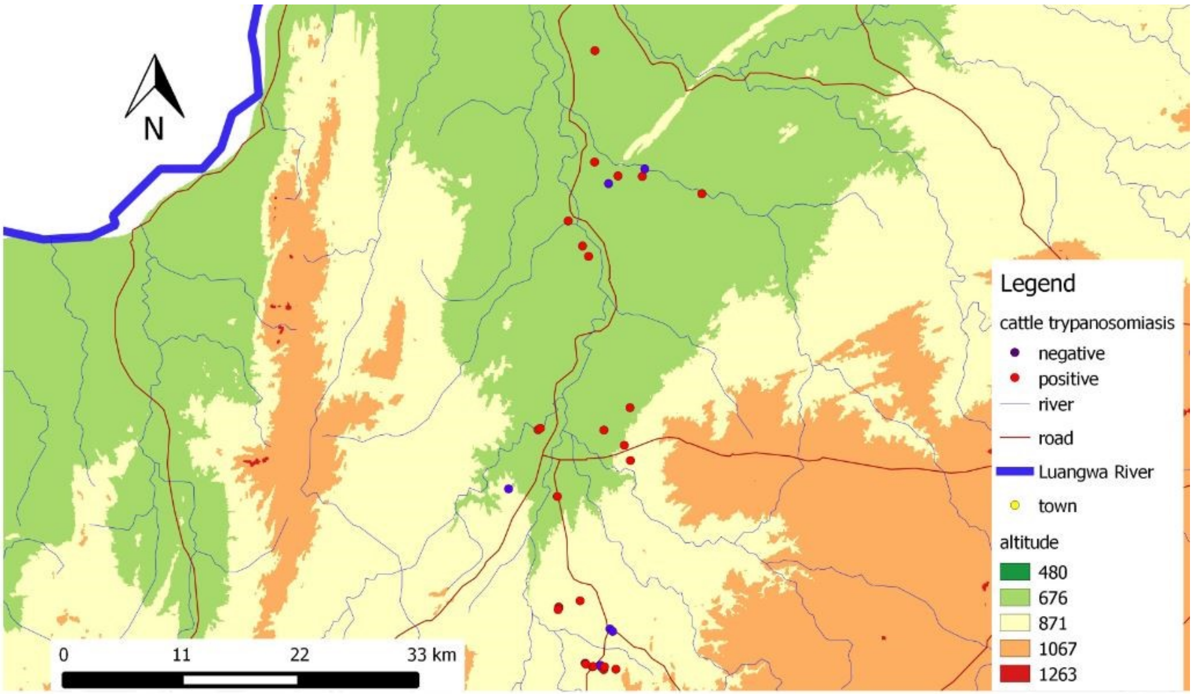


Figure 3-29 Distribution of cattle keeping households testing positive and negative for trypanosomiasis in cattle

The sex of the animal did not have a significant effect on the presence of trypanosomes in the blood (p=0.1176) with 18% (30/163; 95% CI 12.8-25.2) of female and 26% (46/177; 95% CI 19.7-33.1) of male cattle being infected. Age played a significant role with trypanosome infection in that older cattle show more infections (p=0.037).

There was also no association detected between animals that were treated and trypanosome infection (p=0.4704); 22.1% (33/149; 95% CI 15.8-29.7) of cattle were infected despite treatment compared to 23.5% (31/132; 95% CI 16.6-31.7) of untreated cattle being infected.

The reporting of disease signs did not show any correlation to trypanosome infection ($p=0.5319$). Only 20% (3/15; 95% CI 4.4-48.1) of animals showing signs were infected with trypanosomes. But 24% (69/287; 95% CI 19.2-29.4) of animals without any visible signs showed infection in the blood. Interestingly, a remarkable amount of interviewers' comments mentioned that the animal needs dipping or deworming, so there must have been a reason to make this recommendation.

Finally, there was also no significant association between BCS and trypanosome infection in cattle ($p=1$) using Fisher's exact test. For this calculation the animals were put into two BCS groups; the lower five categories (L-M) in one group and the higher four categories (M+-F+) in another group and all trypanosome species occurring in cattle were considered together.

Looking at the influence of traction on nagana in cattle, there is no significance ($p=0.1885$) with 28.9% (26/90; 95% CI 19.8-39.4) of animals used for traction being positive and 21.8% (46/211; 95% CI 16.4-28) of animals not used for traction being positive.

3.3.2.2 Trypanosomiasis in goats

Goats sampled for blood were kept in 67 households. A distribution of age and sex of the samples is shown in Figure 3-30.

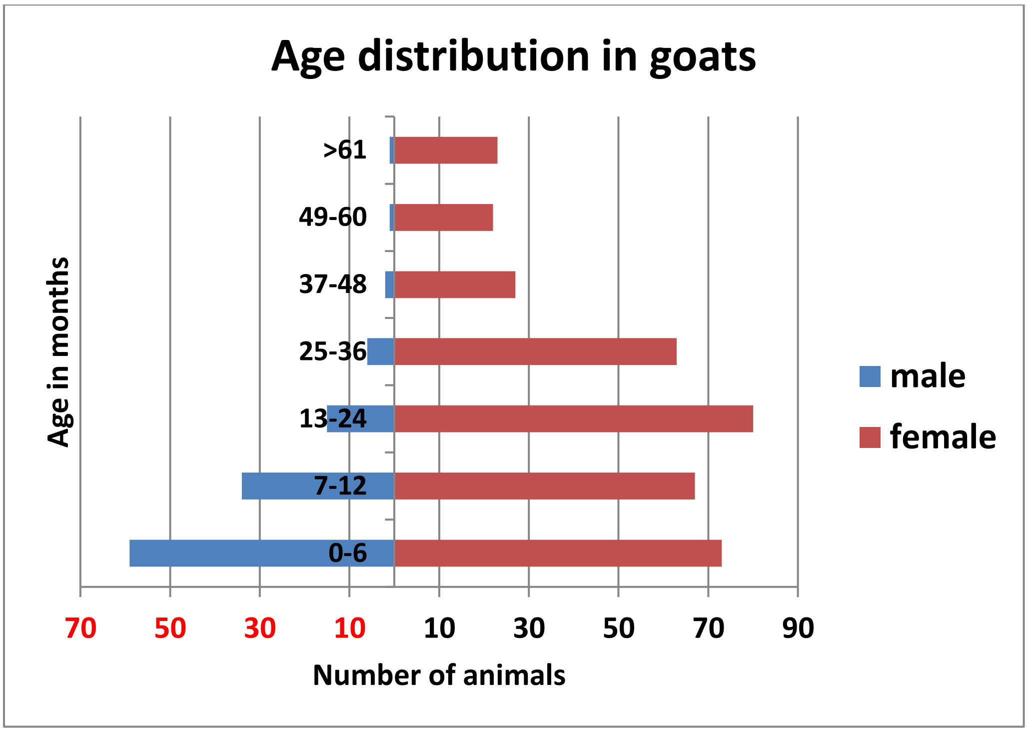


Figure 3-30 Goat population sampled (n=473)

Out of a total of 473 samples, 6.6% (31/473) of samples tested positive for trypanosomes. From the positive samples, 84% (26/31) were single infections, 13% (4/31) were double infections and 3% (1/31) were infected with three trypanosome species. The prevalence of each trypanosome parasite was as follows; *T. congolense* 1.3% (95% CI 0.1-2.4, DE 1.3), *T. congolense Kilifi* 0.2% (95% CI -0.2-0.6, DE 1), *T. vivax* 4.9% (95% CI 2.4-7.3, DE 1.5), *Trypanozoon* 0.8% (95% CI 0.03-1.7, DE 0.9), *T. godfreyi* 0.2% (95% CI -0.2-0.6, DE 1), *T. simiae* 0.2% (95% CI -0.2-0.6, DE 1) and *T. simiae tsavo* 0.2% (95% CI -0.2-0.6, DE 1).

The distribution of households with infected goats and trypanosome free households is shown in Figure 3-31.

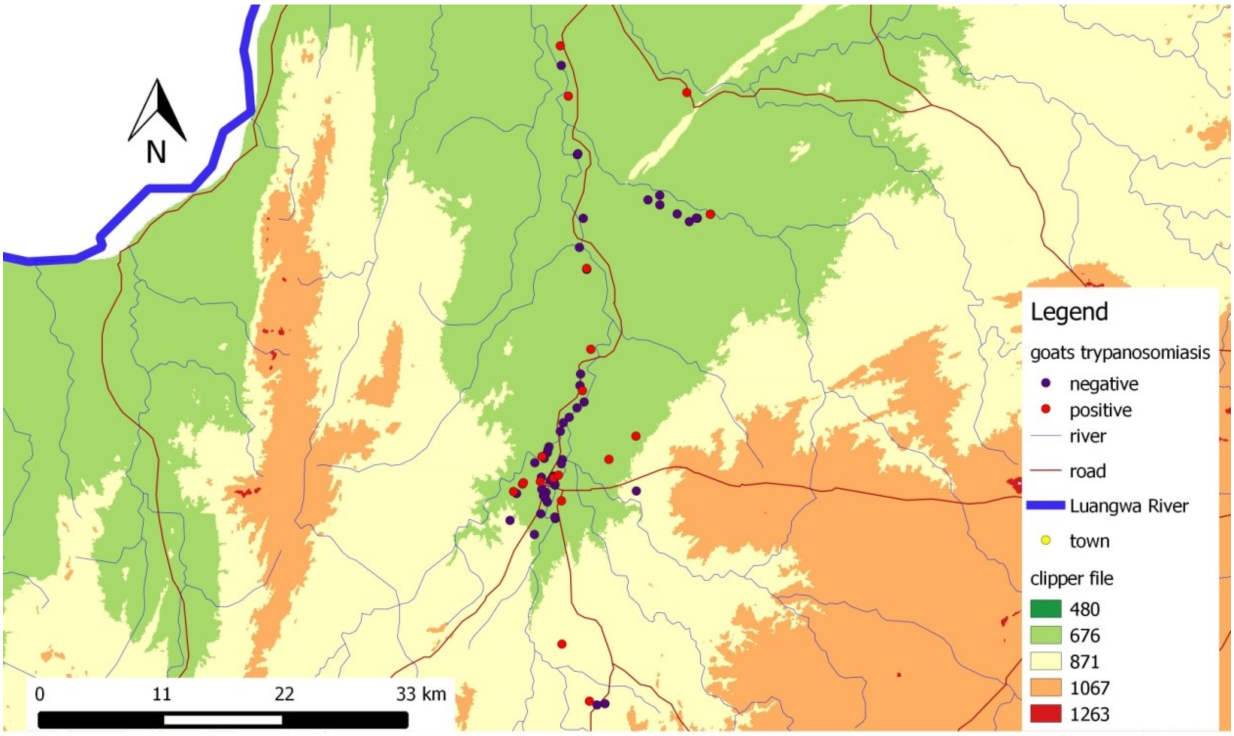


Figure 3-31 Distribution of goat-keeping households testing positive and negative for trypanosomiasis in goats

Although in absolute numbers female goats were with 6.8% (24/355) more often infected with trypanosomes than male goats with 5.9% (7/118), the difference was not significant (p=0.8335). None of the animals were treated or showed clinical signs. Also the BCS (p=0.5574) and age (p=0.071) did not show any correlation with trypanosome infection.

3.3.2.3 *Trypanosomiasis in sheep*

A total of 22 sheep from three households were sampled for trypanosomiasis. The age distribution is shown Figure 3-32.

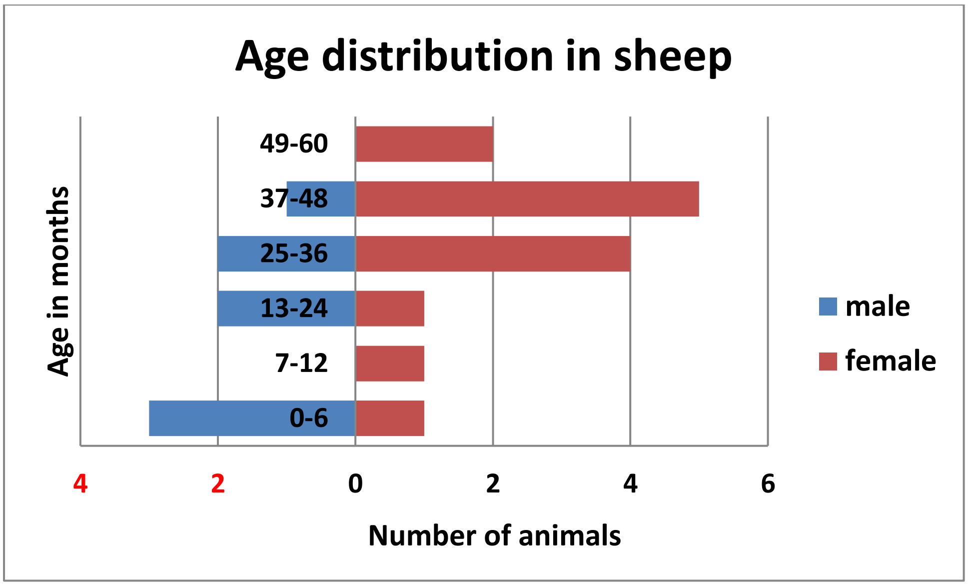


Figure 3-32 Sheep population sampled (n=22)

None of the samples showed up positive during laboratory analysis.

3.3.2.4 Trypanosomiasis in pigs

Pigs were sampled in 46 households. The age and sex distribution can be seen in Figure 3-33.

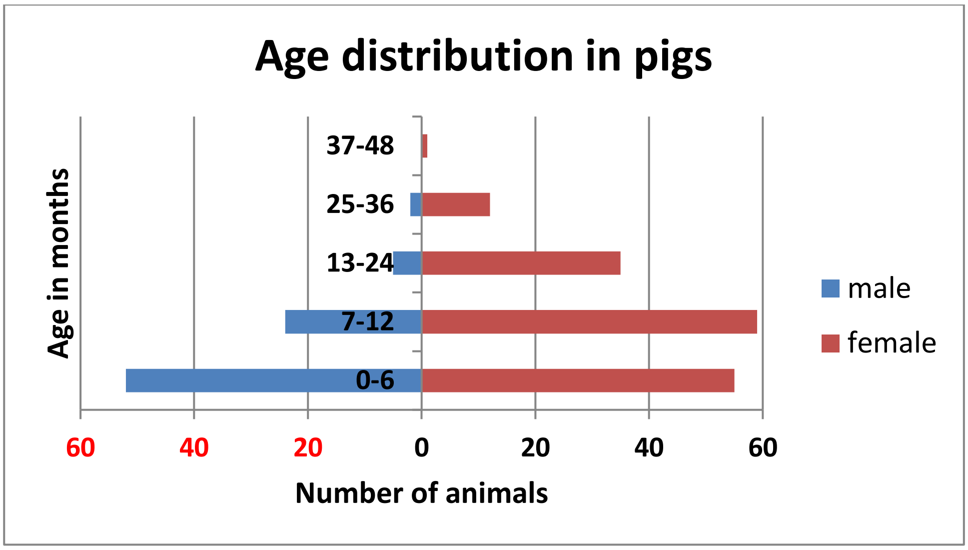


Figure 3-33 Pig population sampled (n=245)

Out of a total of 245 blood samples, 5.7% (14/245) were single infections and the majority of 94.3% (231/245) showed up negative. The trypanosome species prevalence is as follows; *T. congolense* 1.2% (95% CI -0.6-3, DE 1.6), *T. congolense Kilifi* 0.4% (95% CI -0.4-1.2, DE 1), *T. vivax* 2% (95% CI 0.4-3.7, DE 0.8), *Trypanozoon* 0.8% (95% CI -0.3-1.9, DE 0.9), *T. godfreyi* 0.4% (95% CI -0.4-1.3, DE 1.1) and *T. simiae Tsavo* 0.8% (95% CI -0.3-1.9, DE 0.9).

The spatial distribution of pig-keeping households with positive and negative animals is shown in Figure 3-34.

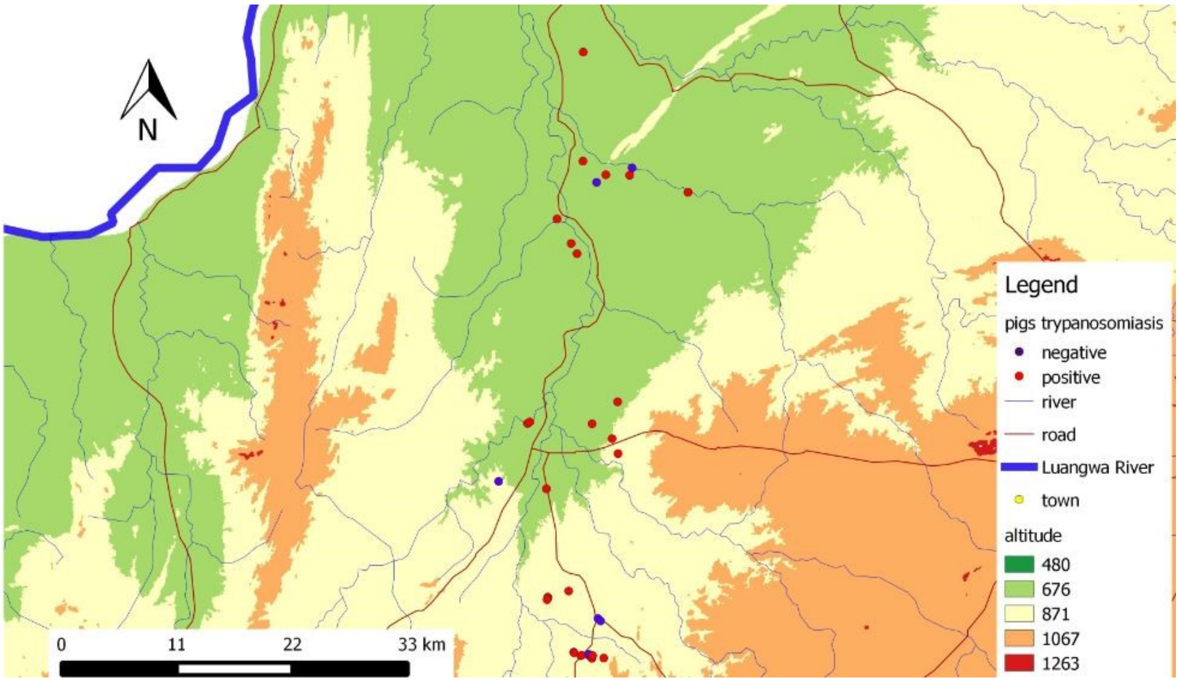


Figure 3-34 Distribution of pig-keeping households testing positive and negative for trypanosomiasis in pigs

Sex (p=0.1493) or age (p=0.071) of the animals did not have any influence on occurrence of infection. Also, if animals showed signs (p=1) or were treated (p=0.0569) did not show any association with trypanosomiasis prevalence. The only significance given was for the relationship between BCS and trypanosomiasis (p=0.0092).

3.3.2.5 Trypanosomiasis in dogs

Blood was taken from dogs living in 70 households. A distribution of age and sex can be seen in Figure 3-35.

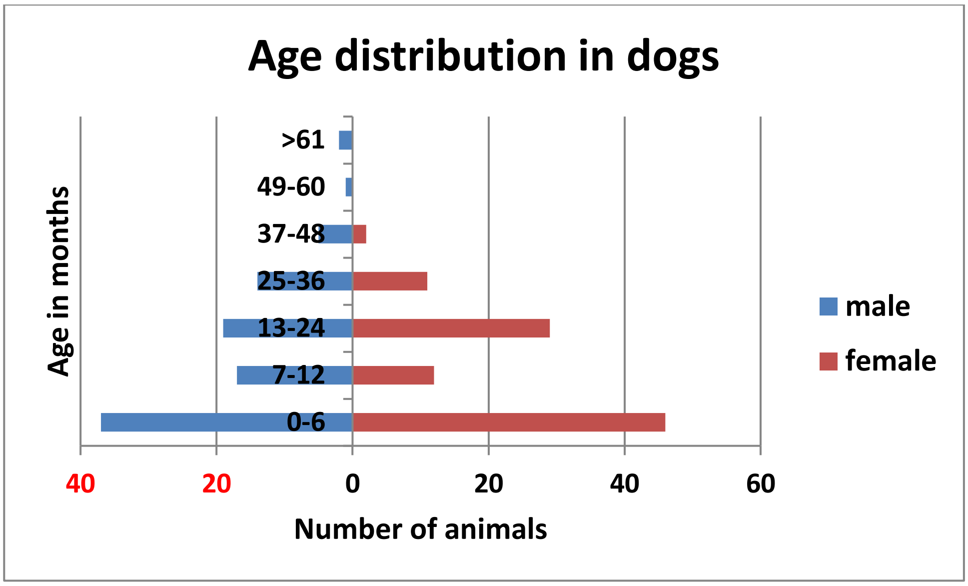


Figure 3-35 Dog population sampled (n=195)

Below is a map showing the spatial distribution of dog-keeping households with positive and negative samples (Figure 3-36).

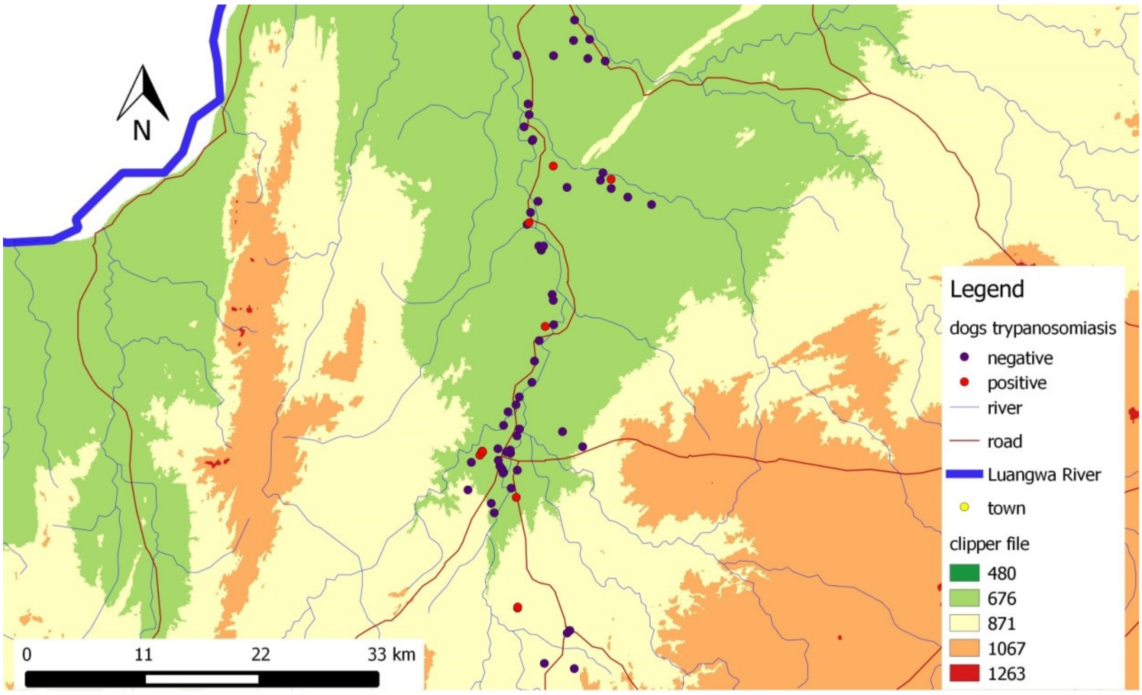


Figure 3-36 Distribution of dog-keeping households testing positive and negative for trypanosomiasis in dogs

A total of 195 samples were tested for trypanosomiasis. The majority with 91.3% (178/195) of samples were found negative for trypanosomes. There were 7.2% (14/195) of dogs with a single infection and 1% (2/195) with a double trypanosome infection and 0.5% (1/195) of dogs found positive for four trypanosome species. The trypanosome species prevalence are for *T. congolense* 1.5% (95% CI -0.2-3.3, DE 1), *T. congolense Kilifi* 1.5% (95% CI -0.7-3.8, DE 1.7), *T. vivax* 1% (95% CI -0.4-2.5, DE 1), *Trypanozoon* 1% (95% CI -0.4-2.5, DE 1) and *T. godfreyi* 4.1% (95% CI 0.1-8.1, DE 2). Although emaciation is a well-known sign for trypanosomiasis (Figure 3-37), the observed BCS did not have a significant influence on trypanosomiasis (p=0.4513).

Figure 3-37 Emaciation can be a sign of trypanosomiasis

Similarly, neither sex (p=0.4514) nor treatment (p=1) did show significance. Even the signs observed did not show a significant correlation with the prevalence of trypanosomes (p=0.6994). Age however showed a significant correlation (p=0.017) with growing number of infections in older dogs.

3.3.3 Tick-borne disease results

Tick-borne infections were only tested on cattle and dog samples, because the microorganism range on the membrane (Table 3-12) was most suitable for these two species. A total of 535 animal samples were tested for TBI; 340 cattle samples and 195 dog samples. The age distribution of the animals tested can be seen in Figure 3-38. Additionally, all wash controls showed negative results and make a contamination between the samples unlikely.

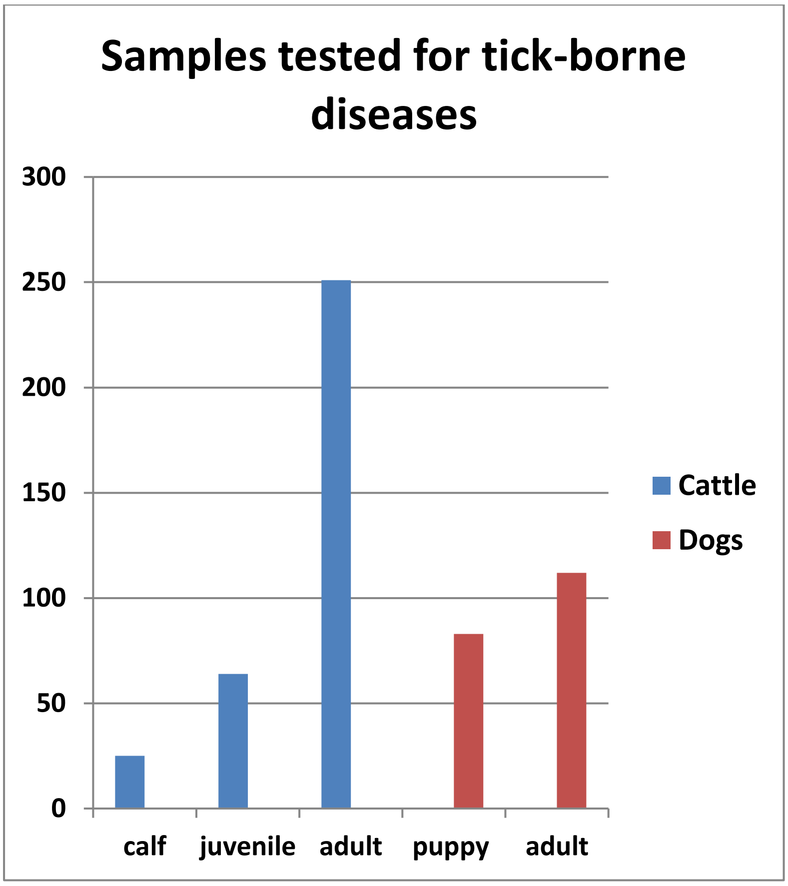


Figure 3-38 Samples tested for tick-borne diseases (n=535)

Figure 3-39 is an example of a result sheet from an RLB. Each black spot shows a positive result for a specific microorganism (row) and a selected sample (column). The 'thickness' of the spot shows a relation to the parasitemia in the blood.

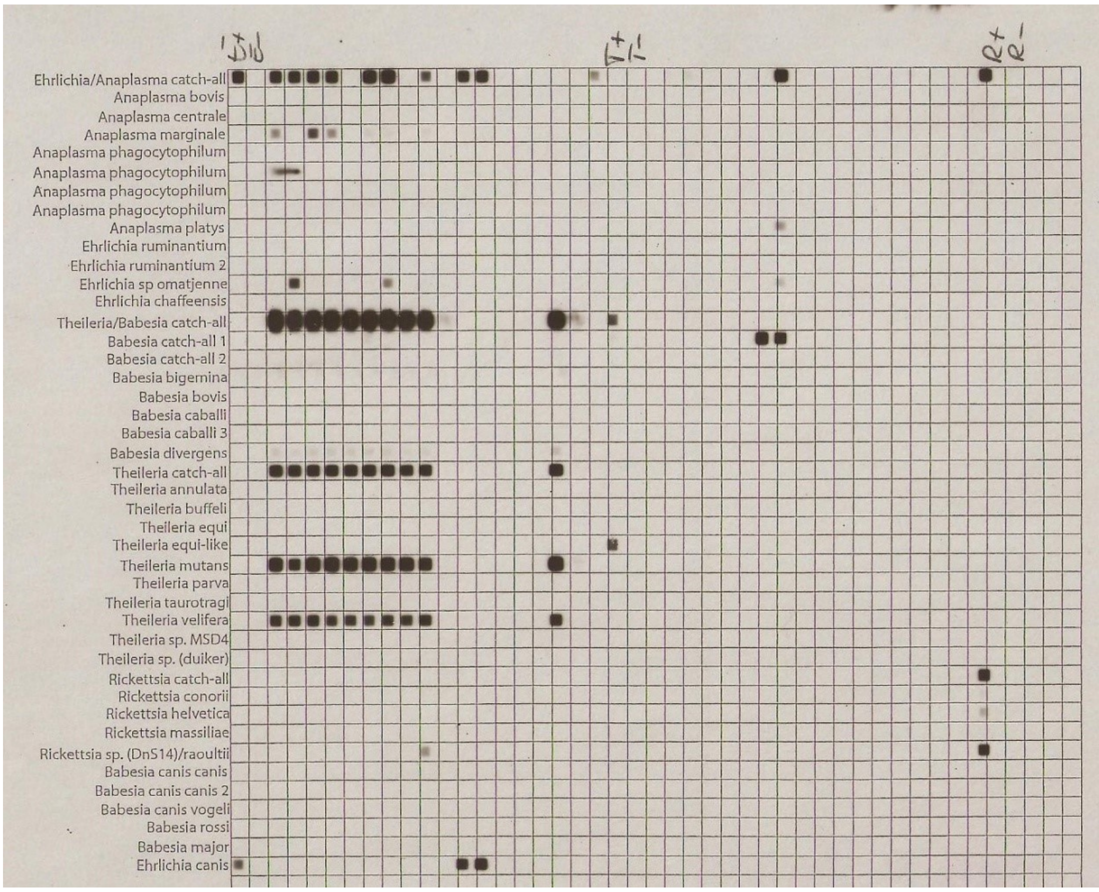


Figure 3-39 Result sheet of an RLB

Ticks were regularly found on the body of the animals as can be seen in Figure 3-40. Detailed numbers are given in the section for each species.

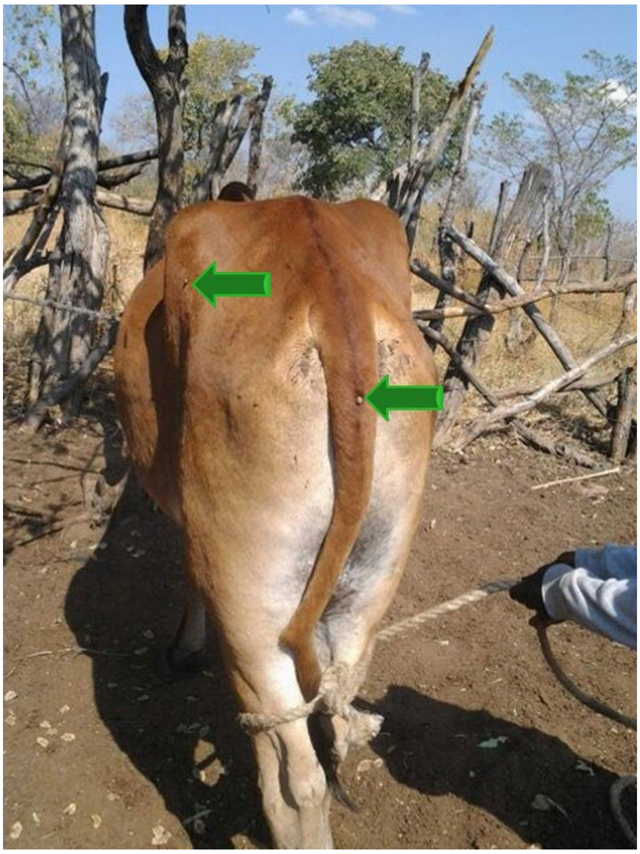


Figure 3-40 Cow with ticks marked by green arrows (taken from animal health questionnaire)

The ability of taking pictures through the use of a digital questionnaire was much appreciated.

3.3.3.1 Tick-borne microorganisms found in cattle

A total of 91.5% (311/340) of samples showed positive results for tick-borne infections. However, at the current knowledge level, not all infections can be considered pathogenic. In Figure 3-41, all cattle keeping households showing positive results for TBI are shown with red spots. Those that do not own infected animals are presented with blue spots.

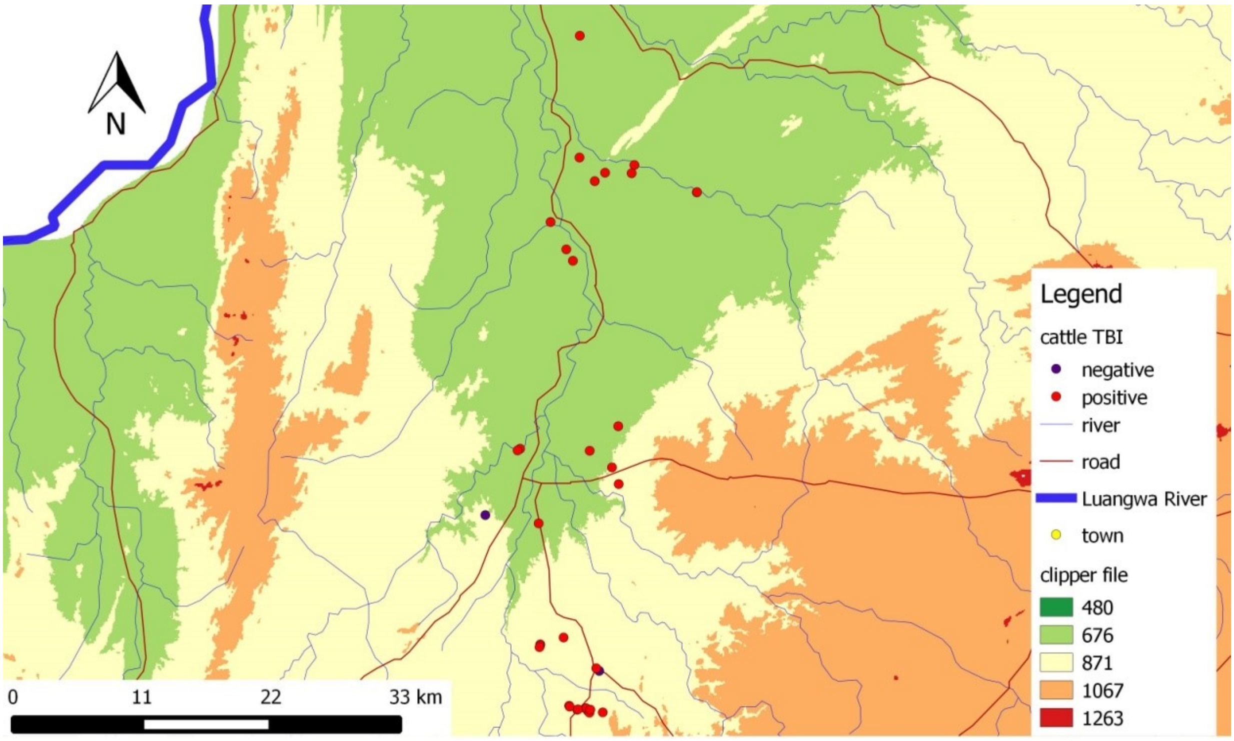


Figure 3-41 Distribution of cattle-keeping households testing positive and negative for TBI in cattle

A detailed overview of the prevalence of selected microorganism in cattle blood can be found in Table 3-20. The design effect presents the ratio of the variance of a clustered sample's estimate to the estimate's variance under simple random sampling . These results can however not be summed up to an overall prevalence, since there are many multiple infections. These will be discussed in 3.3.4.

Table 3-20 Tick-borne infections found in cattle; negative lower confidence interval limits were corrected to 0

	cattle				
	absolute number of samples	**prevalence (in %)**	**lower CI limit**	**upper CI limit**	**design effect**
A/E CA	159	46.8	35.9	57.6	3.9
A. bovis	1	0.3	0	0.9	1.0
A. marginale	50	14.7	8.5	20.9	2.5
A. phagocytophilum	4	1.2	0	3.1	2.5
A. platys	0	-	-	-	-
E. ruminantium	1	0.3	0	0.9	0.9
E. ruminantium new	1	0.3	0	0.9	1.0
E. sp. Omatjenne	17	5.0	0	10.6	5.4
T/B CA	299	87.9	81.3	94.6	3.4
B CA 1	25	7.4	2.9	11.8	2.4
B CA 2	1	0.3	0	0.9	1.0
B. bovis	6	1.8	0.1	3.4	1.3
B. caballi	0	-	-	-	-
B. divergens	34	10.0	0.9	19.1	7.5
T CA	290	85.3	77.6	93.0	3.9
Th. mutans	294	86.5	79.6	93.4	3.3
Th. velifera	215	63.2	54.7	71.7	2.5
R CA	1	0.6	0	1.5	1.1
R. sp. (DnS14)/ raoulti	3	0.9	0	1.9	0.9
R. africae	0	-	-	-	-
B. c. vogeli	0	-	-	-	-
E. canis	1	0.3	0	0.9	1.0

The sex of cattle did not show correlation with TBI (p=0.3318). There were 6.7% (11/163) of female and 10% (18/177) of male cattle showing clinical signs of infection. Also treatment (p=0.8340) did not have an effect. However age played a significant role for TBI (p=0.011).

14.8% (46/313) had ticks on their body when the survey was conducted. Looking at these animals and their infection status, there was no association found (p=0.5487).

95% (41/43) of animals where ticks were found showed positive test results, but also 91.5% (237/259) where no ticks were found showed positive results.

Disease signs similarly do not show any correlation with TBI ($p=0.3383$); 87% (13/15) of cattle with disease signs were RLB-positive, as well as 92% (265/287) of cattle without disease signs. It was also not helpful to take the BCS as an indicator for infection ($p=0.2731$).

Again looking at the influence of traction on TBI in cattle, there is no significance ($p=0.397$) with 90% (81/90) of animals used for traction being positive and 92.9% (196/211) of animals not used for traction being positive.

3.3.3.2 Tick-borne microorganisms found in dogs

Of all dogs, 25% (49/195) were positive for TBI. A map showing the infectivity distribution of TBI in dogs is shown in Figure 3-42.

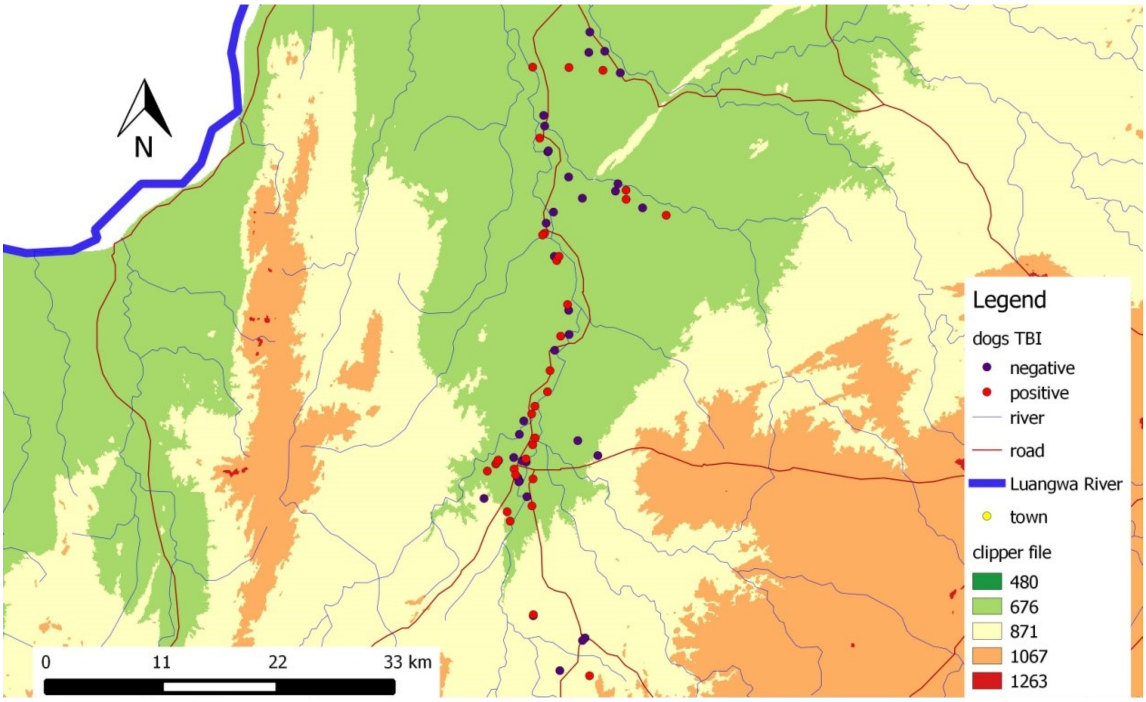


Figure 3-42 Distribution of dog-keeping households testing positive and negative for TBI in dogs

The prevalence of each tick-borne microorganism can be found in Table 3-21. However, there were multiple infections as well so that it is not possible to sum up the individual prevalence.

Table 3-21 Tick-borne infections found in dogs, negative lower confidence interval limits were corrected to 0

	dogs				
	absolute number of samples	**prevalence (in %)**	**lower CI limit**	**upper CI limit**	**design effect**
A/E CA	41	21.0	15.1	25.9	0.9
A. bovis	0	-	-	-	-
A. marginale	1	0.5	0	1.5	1.0
A. phagocytophilum	0	-	-	-	-
A. platys	1	0.5	0	1.5	1.0
E. ruminantium	0	-	-	-	-
E. ruminantium new	1	0.5	0	1.5	1.0
E. sp. Omatjenne	2	1.0	0	2.5	1.0
T/B CA	10	5.1	1.3	8.9	1.5
B CA 1	11	5.6	1.7	9.5	1.4
B CA 2	0	-	-	-	-
B. bovis	0	-	-	-	-
B. caballi	1	0.5	0	1.5	1.0
B. divergens	0	-	-	-	-
T CA	2	1.0	0	2.5	1.0
Th. mutans	3	1.5	0	3.3	1.0
Th. velifera	1	0.5	0	1.5	1.0
R CA	1	0.5	0	1.5	1.0
R. sp. (DnS14)/ raoulti	0	-	-	-	-
R. africae	1	0.5	0.0001	0.0300	-
B. c. vogeli	3	1.5	0	3.3	1.0
E. canis	4	2.1	0	4.6	1.5

One dog was positive for four *Rickettsia* species on the RLB membrane, but despite several sequencing attempts, only one *Rickettsia* species could be identified and therefore it was counted as being positive only for this one. There is however a possibility that the sample contained a *Rickettsia* species not available on the membrane and therefore showed up positive for several.

Sex (p=1), age (p=0.423) and treatment (p=0.0588) did not show a significant association with the occurrence of TBI. The prevalence was similar for female and male dogs with 25% (25/100) and 24.2% (23/95) respectively. The infection rate increased with age from 16% (8/50) as a puppy to 28% (22/58) as a dog with more than 14 months of age. BCS (p=0.3912) and signs (p=0.7858) also did not show any significance. 11% (20/185) of dogs showed signs of disease but this could not be related to TBI.

3.3.3.3 Sequencing results

Unfortunately, not all samples sent for sequencing yielded results. Altogether 10% of samples that did not show up positive for any species-specific probe were sent for sequencing. From 13/132 samples tested for *Anaplasma/Ehrlichia* five samples brought up a usable sequence; when blasted, one showed up as *A. marginale* with 94% query identity and the four others showed up as *A. phagocytophilum* with 97% query identity. The only sample tested for *Theileria/Babesia* yielded no result and from the two tested for *Babesia* alone, one showed a positive result for *Th. mutans* with 97% query identity. From the two samples tested for *Rickettsia,* one showed up as *R. africae* with 99% query identity. These results were incorporated into the analysis together with the other species-specific results.

It should be noted that *R. africae* is not incorporated on the membrane and was therefore not tested for in other samples. In contrast, *A. phagocytophilum* is incorporated with four different probes on the membrane, but it was not detected in this survey using the RLB alone.

3.3.4 Multiple infections

The presence of multiple infections in an animal can have a number of different consequences; it can for example hide concurrent infections in diagnostics (Claridge *et al.*, 2012) or it can prevent infection of a more severe pathogen (Woolhouse *et al.*, 2015).

A table illustrating multiple infections in goats is shown in Table 3-22. Most combinations would include *T. vivax* which was found to be the most prominent trypanosome species in this study.

Table 3-22 Multiple infections in goats

	T. congolense	**T. congolense kilifi**	**T. vivax**	**Trypanozoon**	**T. godfreyi**	**T. simiae**	**Number of parasite species**
	1		1				2
			1			1	2
		1		1			2
			1	1			2
	1		1		1		3
Total	2	1	4	2	1	1	

No multiple infections were found in pigs. Table 3-23 illustrates multiple trypanosome infections in cattle, not taking into account the TBI. Cattle did not have more than two concurrent trypanosome infections and most often it included *T. vivax* and secondly, *T. congolense*.

Table 3-23 Multiple trypanosome infection in cattle

	T. congolense	T. congolense kilifi	T. vivax	Trypanozoon	T. godfreyi	Number of parasite species
			1	1		2
	1		1			2
	1		1			2
		1			1	2
	1			1		2
		1	1			2
	1		1			2
Total	4	2	5	2	1	

Interestingly, the multiple trypanosome infections in dogs do not show *T. vivax* but *T. congolense* Kilifi as the main infection (Table 3-24).

Table 3-24 Multiple trypanosome infection in dogs

	T. congolense	T. congolense kilifi	Trypanozoon	T. godfreyi	T. simiae tsavo	Number of parasite species
	1	1				2
		1	1	1	1	4
	1	1				2
Total	2	3	1	1		

A table showing multiple TBI in dogs is displayed below. Quite a few of these infections could not be uniquely identified as one species, but are shown in the group of *Ehrlichia/ Anaplasma* catch all.

Table 3-25 Multiple tick-borne infections in dogs

	Ehrlichia/ Anaplasma catch all	**Theileria/ Babesia catch all**	**A. marginale**	**E. sp. Omatjenne**	**Th. mutans**	**Th. velifera**	**E. ruminantium**	**E. canis**	**A. platys**	**Babesia catch all**	**R. africae**	**B. caballi**	**B. canis vogeli**	**Number of microorganism species**
	1	1												2
	1	1												2
			1	1	1	1								4
							1	1						2
				1					1	1				3
	1										1			2
								1				1	1	3
	1									1				2
	1												1	2
	1				1									2
Total	6	2	1	2	2	1	1	2	1	2	1	1	2	

If we now look at multiple TBI and trypanosome infections, it becomes obvious that *Ehrlichia/ Anaplasma* catch all is still of considerable importance, but *T. congolense* kilifi is not (Table 3-26).

Table 3-26 Multiple trypanosome and tick-borne infections in dogs

	Ehrlichia/ Anaplasma catch all	**Babesia catch all**	**T. godfreyi**	**T. simiae**	**T. congolense**	**T. congolense kilifi**	**Number of microorganism species**
		1	1				2
	1		1				2
	1			1			2
	1				1	1	3
Total	3	1	2	1	1	1	

There were altogether 70 cattle samples showing multiple infections for trypanosomes and tick-borne mircroorganisms. 67 of them had *Th. mutans*, 49 had *Th. velifera* and 47 had *T. vivax* involved. All other microorgansism were much less frequent. A table with the most frequent combinations of multiple infections in cattle is shown in Table 3-27.

Table 3-27 Most frequent multiple trypanosome infection and TBI in cattle (n=53)

Number of samples positive	T. congolense	T. congolense kilifi	T. vivax	Ehrlichia/ Anaplasma catch all	A. marginale	E. sp. Omatjenne	B. divergens	Th. mutans	Th. velifera	Number of microorganism species
11			1					1	1	3
9	1							1	1	3
9			1	1				1	1	4
7			1					1		2
4			1	1				1		3
3			1		1			1	1	4
2		1			1			1	1	4
2			1			1	1	1	1	5
2			1				1	1	1	4
2	1				1			1		3
2	1			1				1	1	4

3.3.5 Brucellosis

Brucellosis seroprevalence was tested in four animal species; goats, cattle, dogs and sheep. The aim was to test only animals that were sexually mature. Therefore farmers were asked about each animal if it was already sexually active. Additionally, animals that were too young biologically to reproduce were excluded during data analysis. This added measure was performed to avoid counting in any erroneous samples. A species and sex distribution can be seen in Figure 3-43.

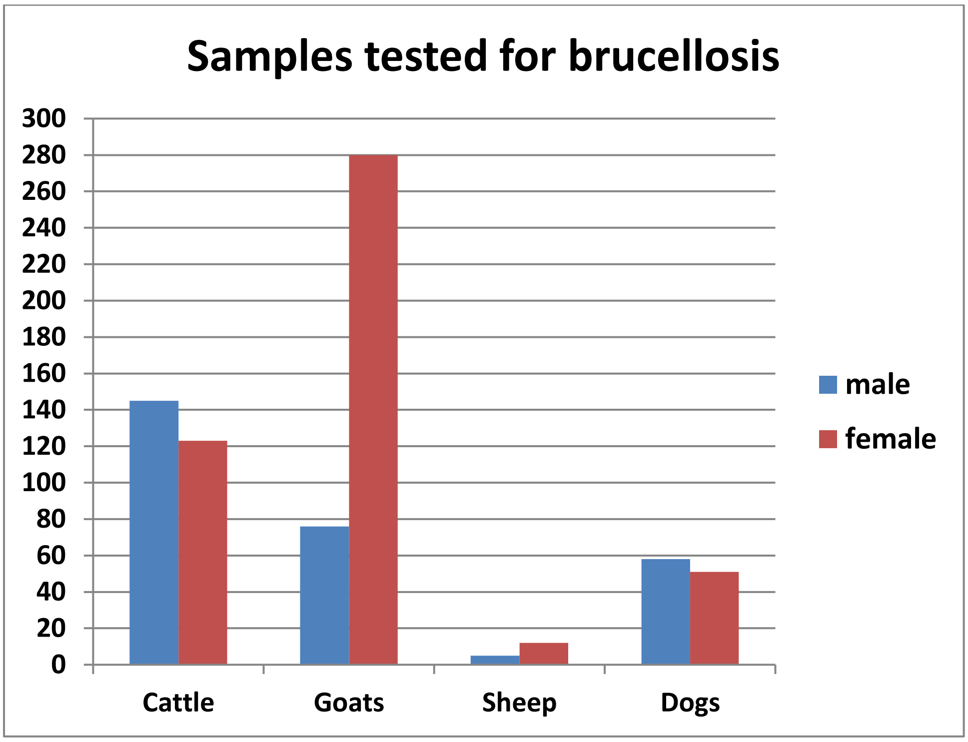


Figure 3-43 Samples tested for brucellosis (n=750)

Altogether 750 animals were tested, of which 737 animals were age-wise correctly tested. The map in Figure 3-44 shows the geographical distribution of the brucellosis samples taken from all animals.

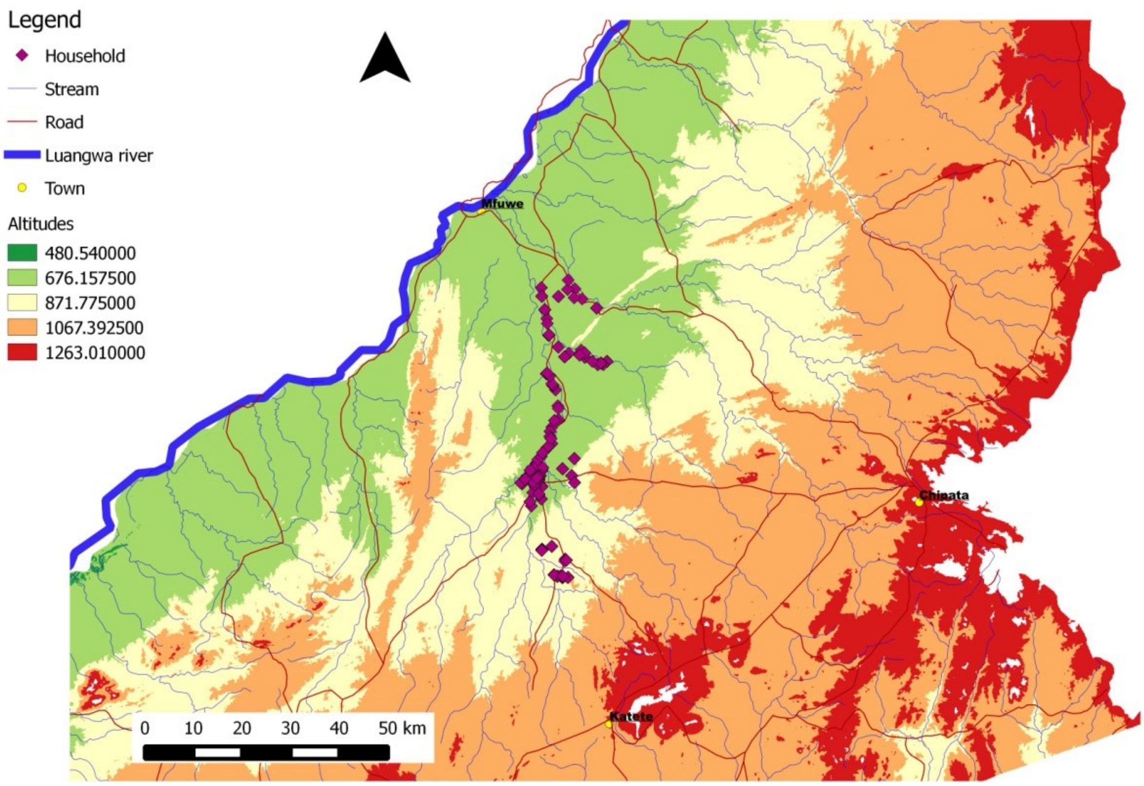


Figure 3-44 Map with the households tested for brucellosis

Detailed results per species are shown in the sections below.

3.3.5.1 Goats

Goats were considered sexually mature when older than 4 months. Altogether 356 goats in 65 households were tested. Out of these, 354 were tested correctly due to age. The sex distribution was 76 samples from male goats and 278 samples from female goats. All samples showed negative results.

3.3.5.2 Cattle

Cattle are acknowledged as sexually mature animals once they reach more than 12 months of age. Therefore out of 268 tested cattle in 33 households, 266 animals were tested correctly. These consisted of 143 male and 123 female individuals. All samples showed negative results.

3.3.5.3 Dogs

Looking at dogs, 101 animals from 61 households were tested. One animal did not have any age identification. Therefore 100 dogs were considered to be tested correctly with more than 7 months of age. 53 were male and 47 female. All test results showed up negative.

3.3.5.4 Sheep

All in all, 17 sheep from 3 households were tested for brucellosis, all at a sexually mature age of more than 5 months. Five animals were male and 12 were female. All tested negative.

3.3.6 Cysticercosis

Many people, not part of this study, approached us during the survey and complained about having fits and epilepsy and that epileptic treatment would not improve the situation. This is not reflected in the wellbeing or health questionnaires, but it suggested that a different disease might be playing a role as a causal agent. It was therefore decided to test for cysticercosis in pigs. Unfortunately, this decision was taken towards the end of the survey. Therefore only eight pigs from four households could be tested. Out of these eight pigs, three pigs from two households tested positive. None of them were positive for tongue palpation.

Figure 3-45 shows the two households with pigs that tested positive.

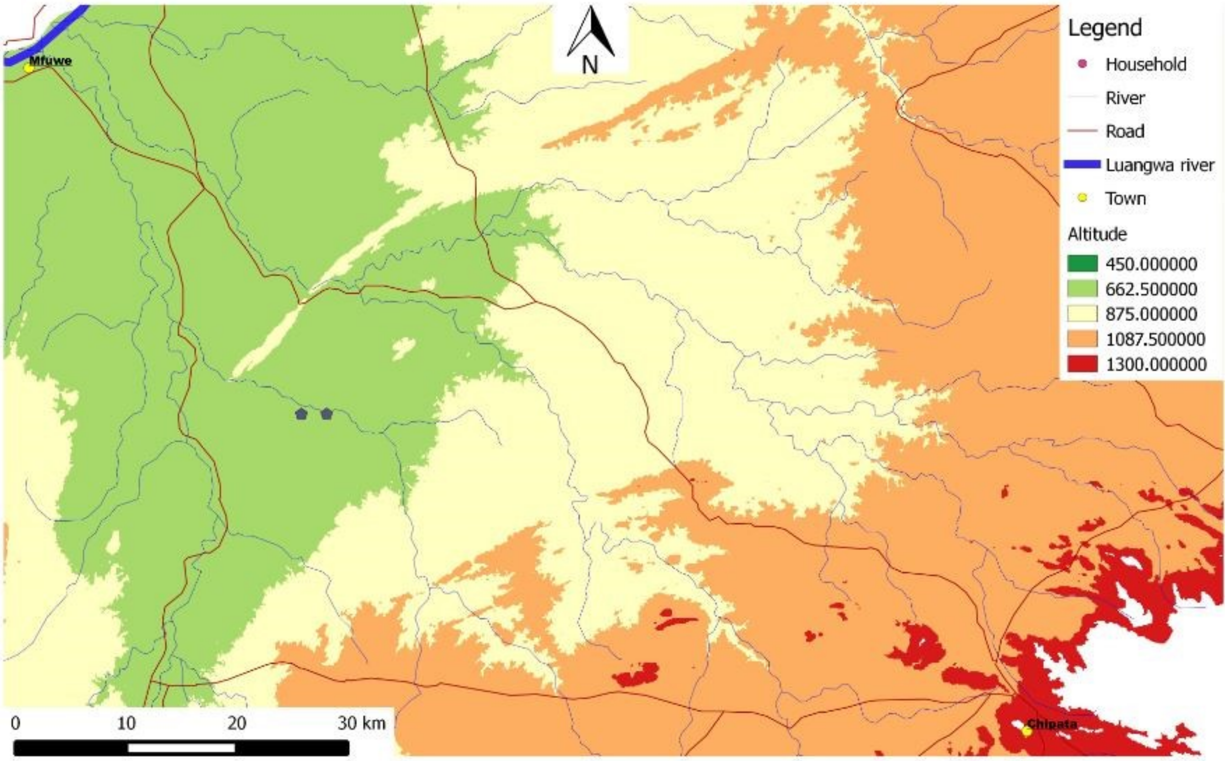


Figure 3-45 Map of households with pigs that tested positive for cysticercosis

In one of the affected households, a blind boy was living. He became blind quite suddenly during early childhood. It would be interesting to know if he was tested positive for neurocysticercosis.

3.3.7 African swine fever

During the census, ASF was mentioned as a major problem in pigs in the area. It is also a notifiable disease in Zambia. It was therefore decided to send samples to the Pirbright Institute in the UK, since it is the international reference laboratory for ASF. Ten samples of pigs showing signs from six households were sent to the laboratory. None turned out to be positive (Figure 3-46).

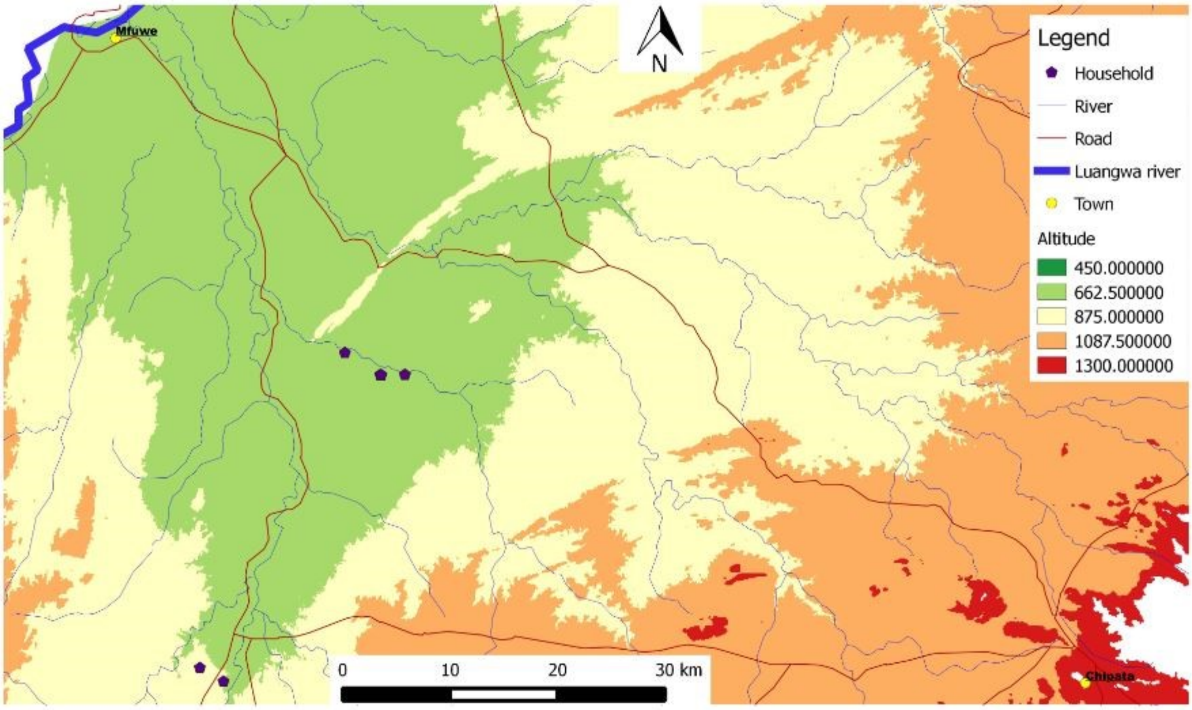


Figure 3-46 Map of households with pigs tested for African swine fever

3.4 Discussion

There are several factors that might have had an influence on the validity of the above presented results. There is the possibility of bias in this study, because not all selected animals could be sampled. Most households keep their animals unrestrained hence it was not always possible to catch all despite of up to three returns to these households. This was especially the case with dogs. However, the number of animals not sampled is very low and this bias should be negligible.

The results from the animal health questionnaire show that there are multiple diseases in animals that could be better managed and it seems that there is a high need of better general veterinary support and drug availability. The age and sex classes were divided into the first class that ends with independence from the mother, the second class of puberty that ends with sexual maturity and then adult animals. In cattle, the adult class is further divided in order to highlight economic factors. In dogs, the adult phase is also divided again. It was reported from a study in Kenya that most dogs would not become older than two years (Kaare and Cleaveland, 1997).

The age and sex distribution in cattle shows roughly in all age classes a ratio of 1:1 which indicates, that there is likely a preference for male traction power (Otte and Chilonda, 2002). An earlier study (Doran, 2000) found that in three out of four of the study sites in Zambia's Eastern Province, half or more of the herd consisted of males, and in one the case where it was less, the ratio was 1.3 in favour of females, still much lower than the 2:1 or higher ratio typically found in pastoral and agropastoral herds where females predominate in order to secure herd growth and high milk production (Otte and Chilonda, 2002). The picture is similar in dogs except in the age class from 3-7 months, which has a ratio of 2:1 in favour of female animals. It is not clear why this is the case. Another study from Kenya showed a male predominance in dog demography (Kitala *et al.*, 2001). Goats and sheep show in general a ratio of 2:1 in favour of female animals increasingly by age. This ratio is different than the one observed in Cameroon of 8:1 (Otte and Chilonda, 2002). Pigs also showed an overall ratio of 2:1 in favour of female animals, but in mature animals alone it was 4:1. This is in contrast to a report from Zimbabwe where the ratio of mature pigs was 9:1 in favour of females (Chiduwa *et al.*, 2008). For the increase in female animals at older age, there could be biological reasons, because herds are better manageable with fewer male than female animals. Furthermore, it is likely that male animals are more often sold or slaughtered and that female animals are kept for reproductive purposes. But due to a lack of market access for the Luangwa Valley, opportunities to sell male animals seem to be lower and therefore the ratio is more even distributed in all species.

Looking at trypanosomiasis, some findings are exceptional. The overall prevalence is low compared to earlier findings (Mubanga, 2008). This might indicate that the situation has become more endemic and less epidemic which again would be explained by less involvement of wildlife as a reservoir (Van den Bossche and Delespaux, 2011). Unfortunately, wildlife testing was not incorporated in this study, so that the current role of wild animals for trypanosomiasis and TBI epidemiology, as compared to earlier studies (Anderson *et al.*, 2011), cannot be clarified. With the unexpected results retrieved from this study, it would be recommended to conduct a wildlife survey in the future. The observed habitat fragmentation could explain lower density of tsetse flies and of wildlife and thus a lower prevalence of trypanosomiasis. Usually ecosystem changes lead to a higher prevalence of diseases, but in this case it could be the opposite.

The nutrition status in most animals was fairly well shortly after the rainy season which might explain why there are not many disease signs related to trypanosomiasis seen. Earlier studies were able to relate the protein intake to the severity of disease (Holmes *et al.*, 2000; Nnadi *et al.*, 2010).

In addition to that, there are only reports of *T. congolense* savannah in the Eastern province in the literature (Masumu *et al.*, 2006b; Masumu *et al.*, 2012). However, this study identified mainly *T. congolense* Kilifi. Furthermore, *T. godfreyi* is primarily found in warthogs and other wild and domestic suids (Auty *et al.*, 2012; McNamara *et al.*, 1994), but this study identified positive samples also for goats, cattle and dogs. *T. godfreyi* was even the species with the highest prevalence in dogs.

The reason for this might be an increased wildlife contact of dogs due to straying or poaching. Similarly, *T. simiae* is usually found only in pigs (Auty *et al.*, 2012; Gibson *et al.*, 2001; Woolhouse *et al.*, 1996), but there were few positive samples in goats in this study as well.

Treatment with diminazene aceturate is also effective against *Babesia* and *Theileria* species (Van den Bossche *et al.*, 2000). Therefore treatment intended against trypanosomiasis might have also reduced prevalence of these two genera.

Trypanosomiasis prevalence in dogs was with 8.7% higher than reported before (5.9%) (Lisulo *et al.*, 2014), but the PCR used in this PhD study is able to detect more *Trypanosoma* species.

To my knowledge, this study is indeed the first to investigate the existence and prevalence of TBI in the Luangwa valley. The risk of ECF and other TBD was well studied on the plateau, but the existence of the vectors was not expected in the valley due to the different climate. This study did not investigate the tick population, but considering the high prevalence of some tick-borne pathogens and the observation of ticks on animals, triggers enough questions for further investigations. In addition, no *B. bigemina* positives were identified. A study from Mozambique suspects that a different probe is needed for this pathogen from this geographic region (Martins *et al.*, 2010).

Especially the TBI samples could not all be identified down to the species level. Selected samples were sent for sequencing, but unfortunately this was not possible for all samples. Therefore the infection pattern could differ slightly from the one presented above.

Tick burden and its related prevalence of TBI are seasonally dependent (Berkvens *et al.*, 1998; Simuunza *et al.*, 2011). The samples of this study were taken 2-4 months after the rainy season which might explain why signs for an acute infection have been less frequent. Additionally, the prevalence for trypanosomiasis and TBI presented here should be expected to be higher in the wet season. A future study on available tick species should be conducted since clarification is needed on the vector of the TBI identified. The role of co-infections between *Trypanosoma* species and TBI also needs further investigation. Cross-immunity seems to be possible (Jongejan *et al.*, 1986; Musisi *et al.*, 1984; Simuunza *et al.*, 2011).

The prevalence of *Anaplasma* species in cattle is similar to that found in Katete district in 2007/2008 (Simuunza *et al.*, 2011). The prevalence of *E. ruminantium* was with 0.3% much lower than in 2007/2008 (5.4%) (Simuunza *et al.*, 2011). The same applies to *B. bovis* with 1.8% now and 10.9% in 2007/2008 (Simuunza *et al.*, 2011). Comparisons between dry and rainy season cannot be done. Furthermore this is the first time that TBI have been tested in dogs in Zambia, thus comparisons to other seasons or years cannot be conducted. It was expected that dogs are infected with tick-borne microorganisms because they have regular wildlife contact and are also close to livestock and owners.

Long term immunization programmes for ECF (Mubamba *et al.*, 2011) could explain why no positive samples for *Th. parva* in cattle could be identified in this study. Concurrent infection of *Th. parva* and *Trypanosoma* species increase ECF mortality (Thumbi *et al.*, 2014) and it would therefore be less likely to find both together in one sample. However, this study found a considerable amount of co-infections. Low pathogenic pathogens are reported to have a protective effect in case of a co-infection (Jonsson *et al.*, 2012; Thumbi *et al.*, 2013; Woolhouse *et al.*, 2015). As already mentioned, in other combinations it can increase mortality (Thumbi *et al.*, 2014; Woolhouse *et al.*, 2015). A future study would have to investigate more on why this is the case and how virulent the current strains are. It would be worth conducting a study of the inter-relationship of tick-borne pathogens and their vectors in the environment of the Luangwa Valley.

The diagnostic method used for ASF is based on antigen detection. It would therefore only be possible to yield positive results, if there was an outbreak. The fact that all tested samples were negative leads to the conclusion that no outbreak was ongoing at the time of sampling. Only one pig was mentioned to be treated against African swine fever by the owner. No details were given for that example. It should be noted however that there is currently no treatment available for ASF and that any protection from the mentioned treatment is doubtful.

This is the first time that domestic animals were tested for brucellosis in the Luangwa Valley. The existence of wildlife was considered to provide a possibility for brucellosis-positivity in the Luangwa Valley as observed in other parts of the country (Muma *et al.*, 2010; Muma *et al.*, 2011). However, samples might have been negative because animals had been treated with penicillin or oxytetracycline before (Banai *et al.*, 2002; Brucellosis, 1986).

The presence of cysticercosis could be confirmed for the first time in the Luangwa Valley. Since this study did not focus on this disease, it is difficult to provide more information, but in neighbouring districts a seroprevalence of up to 17% in pigs and 6% in humans was detected (Mwape *et al.*, 2012; Sikasunge *et al.*, 2008). The zoonotic burden of cysticercosis seems to play an important role in the study area and a more detailed investigation is recommended for the future.

Last but not least, the presence of signs in animals that tested negative suggests that there could be a lack of knowledge and veterinary care or this might hint to another disease not known to be of importance so far. This is supported by the observation that the way animals are kept and handled varies a lot between the different farms. This lack of knowledge could be easily influenced by workshops on livestock and dog management for animal keepers.

3.5 Conclusion

The disease pattern in animal health has not been as expected from former reports. Tick-borne diseases play a major role with several pathogens being present in the area. Cysticercosis seems to be another major problem of zoonotic importance and more research and information is needed. The prevalence of trypanosomiasis has gone down drastically without knowing the causes explaining this. Brucellosis could not be identified from any of the samples. In general, animal production could be improved in the area with relatively simple measures. The interrelationship with the general wellbeing will be discussed in following chapters.

Chapter 4: Survey on disease status in people

This chapter focuses on the human health component of the study conducted in Mambwe District, Eastern Province, Zambia. Blood samples were collected at the same time as the other samples and data. The study describes the infections tested for in people and the prevalence found, the methods applied to study them and the conclusions realised. The study included blood sampling in the field, diagnosis in the field and in the laboratory and human health questionnaires.

4.1 Introduction

This study presents the epidemiology of selected infectious diseases during the time frame of the study. In addition, factors influencing immunity were investigated such as drug abuse and reproductive information.

4.1.1 Aims of the chapter

The main objective was to identify the problems people had and perceived in their health status. The chapter is focused on a survey to investigate sleeping sickness prevalence in humans. Since malaria is usually the first infectious disease suspected and treated for and its signs are initially similar to sleeping sickness, an assessment of its prevalence was also conducted. The aim was also to look at the contributing factors such as body condition score and reproductive status and lists the signs observed and reported in the individual person. The results will be used to identify any changes or developments compared to results of previous studies sourced from available literature.

4.1.2 Selected diseases reported from Zambia

Many diseases play a role in Zambia, amongst them typical health problems in Africa such as HIV/AIDS, but also more rare diseases such as konzo disease which comes from high cassava consumption (Tshala-Katumbay *et al.*, 2013).

A disease with increasing prevalence rates of 1%-53% in Zambia is lymphatic filariasis also called elephantiasis because of the massive swelling of the legs and private parts of infected people. The infection may damage tissue in a way that recovered people need surgery afterwards. The disease is caused by a worm, *Wuchereria bancrofti*. Mass drug administration is the recommended treatment (Kwarteng *et al.*, 2016). Another parasite discussed is *Mansonella perstans*. It can cause swelling too and is by less treatable. For both, microscopy is the gold standard (Simonsen *et al.*, 2011).

In 2015, Zambia also reported an outbreak of bubonic plague in Nyimba District in Eastern Province (ProMED-mail, 2015g). Plague, caused by *Yersinia pestis*, is endemic in several African countries, one of them Zambia. Thirteen people had been affected and three died. Blood samples were collected. Untreated, the case fatality rate can reach 50% for bubonic plague and up to 100% for pneumonic plague. The infection is usually treated with antibiotics (Nyirenda *et al.*, 2017).

Raoult *et al.* report a high prevalence of *Rickettsia africae* in patients returning from southern Africa. The pathogen is zoonotic, transmitted by ticks and causes generally a mild illness with a rash and eschars. The infection can be treated with antibiotics (Raoult *et al.*, 2001).

Epilepsy, probably mainly caused by neurocysticercosis is a well-known problem in Zambia including the Eastern Province where this study was conducted. In the neighbouring Katete district, Mwape *et al.* conducted a longitudinal study to assess the prevalence of cysticercosis. They did three sampling rounds of serology using a monoclonal antibody-based enzyme-linked immunosorbent assay (sero-Ag-ELISA) and a commercial enzyme-linked immunoelectrotransfer blot assay (EITB) (Mwape *et al.*, 2013a). They also took stool samples for taeniosis using coprology and coproantigen ELISA. A seroprevalence of 12.2%-14.5% (sero-Ag) and 33.5%-38.5% (sero-Ab) for the different sampling rounds was found. Taeniosis prevalence was at 11.9% (Mwape *et al.*, 2013a). Interestingly, (re)infection rates as well as seroreversion rates within a year after infection were high. The sero-reversion rates were 44% for sero-Ag and 38.7% for sero-Ab (Mwape *et al.*, 2013a). The global burden of epilepsy is 7.8 million DALYs (Mwape *et al.*, 2013b; Torgerson and Macpherson, 2011). A study conducted in the Northern Highlands in Tanzania reports of a high incidence of epilepsy cases where around 60% of respondents knew of someone in their community with epilepsy (Mafojane *et al.*, 2003). From experience in Latin America, epilepsy is mainly caused by the calcification of cysts, but reports from South Africa suggest that epilepsy is more common in patients with active cysts (Mafojane *et al.*, 2003). This major difference has not been explained scientifically so far.

A survey was conducted in 2007 on intestinal parasite infections in children attending pre-schools in Kafue District in Zambia (Siwila *et al.*, 2010). The results showed a broad spectrum of parasites although the fact that only one stool sample was taken, reduces the sensitivity significantly (Cartwright, 1999). The tests used were Kato-Katz for helminths and a commercial immunofluorescence kit for *Cryptosporidium* and *Giardia*. From 403 samples collected, 17.9% were positive for helminths, 12% had *Ascaris lumbricoides*, 8.3% hookworms, 0.9% *Taenia spp.*, 0.6% *Hymenolepis nena* and 0.3% *Schistosoma mansoni*. In addition a prevalence of 28% was found for *Cryptosporidium* and 29% for *Giardia* (Siwila *et al.*, 2010).

4.1.3 Sleeping sickness in the study area

Trypanosomiasis is caused by a blood parasite that can be classified in several species (Barrett *et al.*, 2003a; Kennedy, 2013). It is called sleeping sickness in humans and nagana in animals, although not all trypanosome species are zoonotic in nature. Trypanosomiasis is a neglected disease and has a high burden depending on the location and prevalence. In Tanzania, a study calculated the DALY estimate between 215.7 and 978.9, either including age-weighting and under-reporting or not (Matemba *et al.*, 2010). The disease-related costs spent for the 143 patients being part of that study were estimated at US$ 15,514 and the indirect non-medical costs at US$ 9,781 (Matemba *et al.*, 2010).

The disease occurs in 36 sub-Saharan African countries and is mainly transmitted by tsetse flies. People coming into frequent contact with tsetse flies, such as farmers, hunters and other people in rural areas, are at a higher risk of contracting the disease. It can affect single villages or an entire region. Very rarely congenital infections have been reported (Ngoma *et al.*, 2004). The prevalence of the disease can vary substantially between the villages within an infected area depending on the environmental conditions (MacLean *et al.*, 2010). Twenty one million people living within the tsetse belt are therefore at moderate to very high risk of exposure to this parasite (Simarro *et al.*, 2012). Figure 4-1 shows the typical type of vegetation where one could find an abundance of tsetse flies.

Figure 4-1 Natural vegetation in the valley with grass, bushes and trees; an ideal habitat for tsetse flies

The overall prevalence of the disease in humans has significantly decreased with a worldwide incidence of below 10,000 reported cases in 2009 for the first time in fifty years, whereas the number of reported cases in 1998 was 40,000 (WHO, 2012a). In 2010 only 7139 new cases were reported, but the number of new cases is currently estimated at 30,000 a year due to underreporting (WHO, 2012a). In the 1960s, the disease was considered to be well-controlled in the known foci in Africa, but a lack of continuity in control measures and surveillance made the prevalence rise again.

There are also great concerns about the proportion of underreported, undiagnosed and therefore untreated cases (Fèvre *et al.*, 2008; Welburn *et al.*, 2015; WHO, 2012a). The problem of underreporting and non-diagnosing is related to the performance of the local and national health system and the health workers involved. A survey assessing the situation for human African trypanosomiasis (HAT) management in Mpika District in northern Zambia had alarming outcomes (Mwanakasale *et al.*, 2013). The HAT knowledge of health workers from ten health institutions was questioned as well as the general capability of response and management of HAT cases assessed. Less than half of the respondents scored more than 50% on the knowledge questionnaire. None of the health workers was able to distinguish the two clinical stages of HAT from each other. Overall there were only three medical doctors in the only bigger hospital in Mpika District. The supply of antitrypanosomal drugs as well as refresher trainings were insufficient. A lack of proper diagnostic capacities and a non-functioning referral system only worsens the situation (Mwanakasale *et al.*, 2013). There are plenty of examples of bad case management of sleeping sickness in the affected countries (Bukachi *et al.*, 2009; Sindato *et al.*, 2008).

Countries suffering the most from epidemic outbreaks of HAT are the Democratic Republic of Congo, Angola and southern Sudan with prevalence up to 50% in certain villages. Zambia reports less than 100 cases per year (WHO, 2012a), and the trend shows a decreasing prevalence over time (Mwanakasale and Songolo, 2011). Between the early 1960s and mid 1990s, the main affected areas were Western, North Western, Lusaka, Eastern, Luapula and Northern Provinces of Zambia. From the 1990s till 2000 intensive vector control programmes were conducted. Between

2000 and 2007, the only districts recording HAT cases were Mpika, Chama and Chipata (Mwanakasale and Songolo, 2011). Infection occurs mainly during rainy season and the proximity to wildlife poses a high risk of getting infected (Dukes *et al.*, 1983). Despite decreasing overall prevelaence, there have been continuous reports of cases also abroad from tourists who went on a safari in Zambia and came back home with an infection of sleeping sickness (ProMED-mail, 2015i; ProMED-mail, 2016b; ProMED-mail, 2017b). Sometimes they were related to a local outbreak (ProMED-mail, 2014e). There were even cases reported from areas where there have been none for several years, so resurgence of the disease, especially in the proximity of wildlife, should be expected (Sindato *et al.*, 2008). A survey conducted in the northern Luangwa Valley found a point prevalence of 5.8 per thousand. None of these cases was symptomatic. Other parasites identified were *Plasmodium* and *Mansonella perstans* (Wurapa *et al.*, 1984a).

The principal cause of sleeping sickness in humans (97%) is the parasite *Trypanosoma brucei gambiense (T. b. gambiense)* (Kennedy, 2013). It is found in west and central Africa and causes a chronic infection, which initially may not show any symptoms for months or years, but when signs emerge, it has often already affected the central nervous system (Kennedy, 2013). In Zambia and other parts of eastern and southern Africa, *T. brucei rhodesiense* (*T. b. rhodesiense*) is the cause of sleeping sickness (Barrett *et al.*, 2003a). Historically, sleeping sickness is widespread in the Luangwa Valley and the Eastern Province of Zambia but cases occur sporadic (Dukes *et al.*, 1983). It causes an acute infection with symptoms appearing earlier than with *T. b. gambiense* and with a rapid progression of the disease into the central nervous system. However, there are reports of sub-acute forms of the disease and even asymptomatic carriers (Buyst, 1977; Mwanakasale *et al.*, 2014; Songa *et al.*, 1991; Wurapa *et al.*, 1984b). These cases resembling more a chronic form of the disease occur more often in southern Africa whereas the disease becomes more severe and acute the further north it occurs (MacLean *et al.*, 2010).

The pathogenesis in humans starts with fever, headaches, joint pains and itching (Kennedy, 2013). Very typical to observe is the Winterbottom's sign shown in Figure 4-2. As soon as the parasite crosses the blood-brain barrier, the neurological phase starts and other symptoms become more obvious: changes of behaviour, confusion, sensory disturbances, poor coordination and disturbance of the sleep cycle. Actually, nearly all case reports mention their patients to sleep during the day and being awake at night which may raise the fear of neighbours against possible bad spirits (Bukachi *et al.*, 2009; Ngoma *et al.*, 2004; ProMED-mail, 2015i; ProMED-mail, 2016b; Sindato *et al.*, 2008). Sleeping sickness is invariably fatal if untreated (Kennedy, 2013).

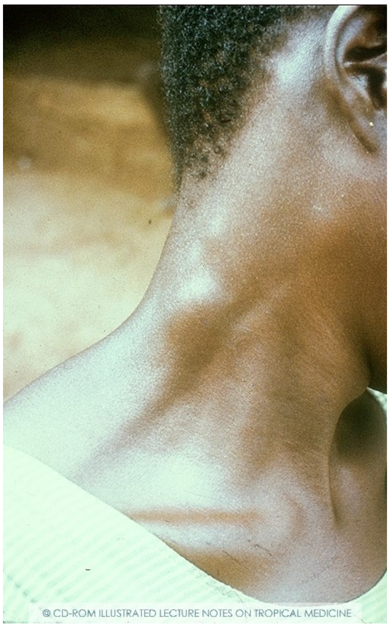


Figure 4-2 Winterbottom's sign: Swollen posterior cervical lymphnodes (Van den Enden)

The diagnosis of the disease usually starts with the onset of symptoms. There are serological tests as well as molecular detection tests like the polymerase chain reaction (PCR) developed (Moser *et al.*, 1989; Namangala *et al.*, 2012; Picozzi *et al.*, 2008). In order to distinguish between first and second stage of the disease a lumbar puncture has to be performed (Kennedy, 2013). Results have to be assessed carefully looking at the whole picture, since cases of continued PCR positivity have been reported in around 20% of successfully treated patients (Deborggraeve *et al.*, 2011). And again the clinical diagnosis is an important component of the correct diagnosis.

A study from Kenya and Uganda revealed that 86% of HAT cases used more than two different healthcare options and 70% more than three different health institutions before getting the correct diagnosis (Bukachi *et al.*, 2009). More than half of the cases suffered more than two months until diagnosed and therefore often received treatment only in the late stage of disease, which is more dangerous for the patient (Bukachi *et al.*, 2009).

Treatment however is expensive and risky, because there are only few medicines available to treat the disease and they are different depending on the stage of infection. The cure rate is best if the disease is diagnosed early due to the difficulty of pharmaceuticals crossing the blood-brain-barrier (Kennedy, 2013). The problem here is that most clinics would not store the drug. Zambia has a national focal person for sleeping sickness drug supply. This person needs to notify WHO to be able to receive the drugs needed (personal communication Malimba Lisulo). This is supposed to help WHO in minimising underreporting, but it leads to significant if not lethal delays of treatment (Bukachi *et al.*, 2009; Ngoma *et al.*, 2004; Sindato *et al.*, 2008). Furthermore, resistance against some of the drugs is increasing. The biggest disadvantages of the treatment are the major side effects of the drugs. Melarsoprol for example, the late stage drug against *T. b. rhodesiense*, can cause death in 5% of patients who develop a post-treatment reactive encephalopathy (Kennedy, 2013; Rodgers *et al.*, 2011). Therefore it has been tried to use an oral treatment with two melarsoprol cyclodextrin inclusion complexes and it looks promising (Rodgers *et al.*, 2011). WHO provides the drugs free of charge to endemic countries. The development and commercial production of new drugs such as eflornithine has been hindered mainly by ignorance from the pharmaceutical companies, but lately improved in a higher awareness of neglected diseases and increased funding research activities for these diseases for example by the Bill and Melinda Gates Foundation (Barrett, 2006).

Sleeping sickness is a disease best tackled using the One Health approach. Even if the parasite in humans was eliminated, the fact that animals are a reservoir means that the infection cycle can start again. Therefore animals and humans have to be treated at the same time (Funk *et al.*, 2013). Many times cattle are mainly targeted for trypanosomiasis control, but a study in Kenya found out that pigs are more likely to carry *T. brucei* s.l. than other livestock which means they should definitely be included in the control management (von Wissmann *et al.*, 2011).

Text box 4-1 Sleeping sickness case in St Francis hospital in Katete

During the survey, the team was informed that a case of sleeping sickness was presented to St. Francis hospital in Katete. The team was able to visit him shortly and interview the mother who was with him. The boy was eleven years old and had been fishing regularly. He started in December 2012 having neurological problems with this leg. He soon went to the health centre where they noticed fever and suspected an infection. He was treated for malaria and also received antibiotics, but did not get better. Several tests were done, but it took until around April/ May that they identified sleeping sickness as the cause of his illness. An earlier test for trypanosomes had been negative, but cerebro-spinal fluid (CSF) testing confirmed the suspicion. The boy was then treated with melarsoprol, a highly toxic drug based on arsenic. When the team was visiting him, he was not responsive, but had shown already some improvement according to the doctor and his mother. This case exemplifies how long a correct diagnosis of sleeping sickness can take although it is a well known infectious disease in the area.

4.1.4 Malaria in the study area

Malaria is a vector-borne diseases caused by *Plasmodium* species and transmitted by *Anopheles* mosquitoes. There are five different species known to affect humans *Pl. falciparum, Pl. malariae, Pl. ovale, Pl. vivax* and *Pl. knowlesi*. *Pl. falciparum* causes the infection with the highest case fatality rate. *Pl. knowlesi* plays an increasing role in South-East Asia and is a zoonotic species, shared usually between humans and monkeys (Brock *et al.*, 2016). According to the World Malaria Report, death rates due to malaria infection have decreased by 47% between 2000 and 2014 (ProMED-mail, 2014c). Global malaria deaths were estimated at 995,000 in 1980, 1,817,000 in 2004 and 1,238,000 in 2010. Except in Africa, malaria cases steadily decreased (Murray *et al.*, 2012). However, there is an alarming increase in drug resistance (ProMED-mail, 2014c). In Mali, a hybrid mosquito resulting from interbreeding of two species was identified as resistant to bed-net insecticide (Norris *et al.*, 2015). The malaria cycle can be seen in Figure 4-3.

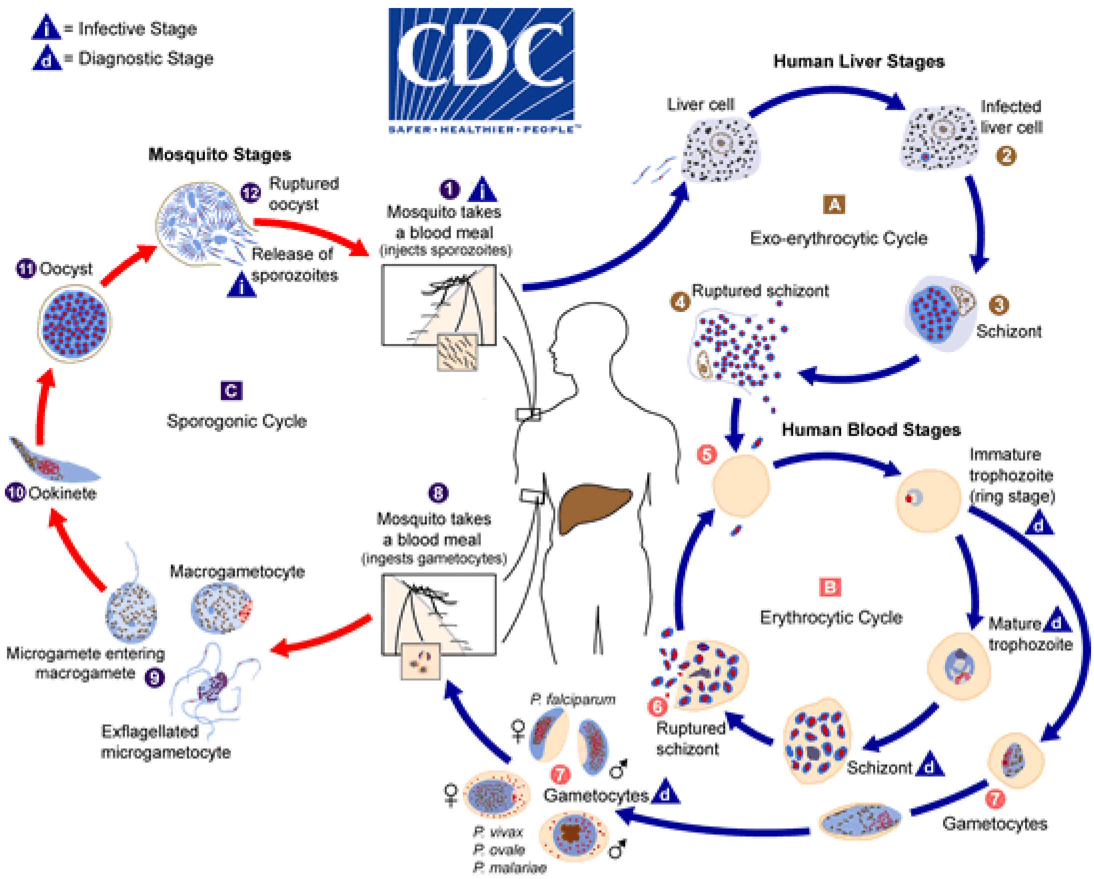


Figure 4-3 Malaria cycle (CDC, 2016)

Malaria shows flu-like symptoms with fever, headache, body ache etc. The fever has a defined pattern depending on the species the person is infected with. In a progressed stage it leads to an enlarged spleen and jaundice due to an impact on the liver. In severe cases, malaria can cause encephalitis and other neurological problems. This leads often to underreporting of rabies cases because the clinical picture is so similar (Mallewa *et al.*, 2007). The infection can reappear after varying symptom-free periods either as recrudescence, relapse, or reinfection. Recrudescence is when parasites surviving in the blood as a result of inadequate or ineffective treatment cause symptoms after a symptom-free period. Relapse is more often seen 8-24 weeks after cure and usually caused by *Pl. vivax* and *Pl. ovale*. It means that the parasites have been eliminated from blood but persist as dormant hypnozoites in liver cells. In contrast to that, reinfection signifies a new infection with the same or another species of *Plasmodium*. It is difficult to distinguish reinfection from recrudescence, but recurrence is easier to diagnose through the time frame. An infection with *Pl. falciparum* during pregnancy can cause a low birthweight which then represents a high risk for neonatal and infant death (Eisele *et al.*, 2012). Therefore malaria prevention during pregnancy is of increased importance (Eisele *et al.*, 2012). People with sickle-cell anaemia are resistant to malaria.

Malaria is considered with such importance because it has a high economic impact (Bhatt *et al.*, 2015; Chima *et al.*, 2003). The way it is usually measured calculating the direct costs of prevention and treatment against the indirect costs of productive labour time lost. However, Chima *et al.* argue that these calculations need to be improved because they do not reflect the burden to society nor the additional benefits from control. Also the uncomplicated malaria is overestimated and the severe or fatal infections are underestimated (Chima *et al.*, 2003). Similar arguments are given by Crowell *et al.* with a need to include for example the whole range of ages of hosts and the seasonality patterns (Crowell *et al.*, 2013). Microeconomic factors associated with malaria were assessed by Coleman et al. (Coleman *et al.*, 2010). They identified in increased risk for people living in mud-walled houses compared with brick dwellings.

The disease occurs mainly in low income countries in the tropical regions, but parts of southern Europe had autochthonous cases during the recent years. Global warming and climate variations are made responsible for the spread to new regions and even to higher altitudes (Siraj *et al.*, 2014). Temperature influences the population growth of the vector but also the pathogen development within the vector (Siraj *et al.*, 2014).

Also land- use change and the impact of people entering new habitats through deforestation like in South East Asia are held responsible for example for the emergence of *Pl. knowlesi* as a fatal human pathogen. This emergence is actually a good example of the complexity and dynamics of the interaction between human activities, animals, parasites and mosquitoes (Brock *et al.*, 2016). It is not clear if human-to-human transmission is possible with this species, but it is well know that it creates such a tremendous outcome because the asexual lifecycle in humans takes only 24 hours and end with a high parasitemia (ProMED-mail, 2014b).

The malaria programme in Zambia is evaluated well. The government prioritizes malaria in their National Health Strategic Plan as well as in the National Development Plan (Chanda *et al.*, 2012b). The strategy includes an integrated vector management with focus on four elements; early and rapid diagnosis and treatment, implementation of sustainable preventive measures including vector control, detection and containment of epidemics and capacity building of local research (Chanda *et al.*, 2008). The successful reduction of malaria in Zambia was also achieved through an increased funding from US$ 9 million in 2003 to US$ 40 million in 2008 (Chizema-Kawesha *et al.*, 2010). A study from 2009 reported a prevalence of 7% using microscopy (Keating *et al.*, 2009). Nevertheless, Shiff *et al.* consider the introduction of passive surveillance for malaria in Zambia as useful. The increased use of RDTs would make that possible and could provide better insights into the dynamics of the disease and lead to reductions in the use of antimalarial drugs (Yukich *et al.*, 2012). So far only a series of cross-sectional studies have assessed the situation (Shiff *et al.*, 2013). The use of mobile phones could even help establish active surveillance for malaria by sending electronic reports of all positive cases in a health centre to the national epidemiology department (Kamanga *et al.*, 2010).

The percentage of households with at least one insecticide treated net (ITN) increased over the years in Zambia from 14% in 2001/ 2002 to 62% in 2008 (Chanda *et al.*, 2012a). The use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) in Zambia has been investigated by Chanda *et al*. They found a significant reduction of mortality and case fatality rates in urban districts using IRS, but not in rural districts using LLINs (Chanda *et al.*, 2012a). The effect of the preventive measures together with artemisinin-based combination therapy (ACT) has to be investigated (Chanda *et al.*, 2012a). In the Eastern Province of Zambia, around 500,000 IRS and 4,000,000 LLINs have been distributed (Kamuliwo *et al.*, 2013). However, the districts in north-eastern Zambia remained malaria hotspots despite the national declines in 2006-2011 (Kamuliwo *et al.*, 2013).

For decades, scientists investigate in developing a good malaria vaccine, but for example the most promising vaccine at the moment called Mosquirix still needs three injections and is therefore not convenient to use in reality. It is however a very promising candidate and would be the first vaccine to protect against a parasite. More research needs to be conducted (Wilby *et al.*, 2012). There is also another candidate vaccine called RTS,S vaccine. It needs four injections to be efficient and is then still less protective than an ITN (Seo *et al.*, 2014). A study in mice highlighted the importance of the gut microbiota for a protective immune response against malaria and may lead to the development of an oral vaccine (Yilmaz *et al.*, 2014).

Livestock keeping can provide protection from malaria for a household (Franco *et al.*, 2014; Jaleta *et al.*, 2016; Mayagaya *et al.*, 2015). Cattle seem to be exceptionally effective in reducing the risk of *Pl. falciparum* infection in southern Zambia, but other animals such as goats, dogs and cats had an protective effect as well (Bulterys *et al.*, 2009). The protection can happen in two ways; one in that mosquitoes are also attracted to the animals and therefore the bites per person are reduced. Secondly, any insecticidal treatment of the animals may have an effect on the mosquito population as well and thus reduce the risk of infection.

Issues using rapid diagnostic tests for malaria are manifold. The histidine rich protein-2 (HRP-2) antigen for example can stay for more than 6 weeks after successfully treating *Pl. falciparum* infection (Gillet *et al.*, 2013). Rapid diagnostic tests are more routinely used for malaria testing now. However, false positivies may happen especially in cases where the patient is infected with HAT (Gillet *et al.*, 2013). The specificity differed among the tests and those that targeted mainly pan-pLDH (lactose dehydrogenase) reported the false positives more frequently (Gillet *et* *al.*, 2013). Comparing microscopy to a RDT, the study by Keating *et al.* also found false positivity in HRP-2 results (Keating *et al.*, 2009). A study conducted by Yeboah-Antwi *et al.* distributed RDTs among community health workers (CHW) to increase the probability of detecting severe malaria in children. At the same time, they were told to judge cases of pneumonia. In non-severe cases they were given treatment and otherwise sent to the nearest health centre. The design used was case-control, where control CHWs would not use RDTs but provide direct treatment for malaria and send pneumonia cases to the clinic. Intervention CHWs used RDTs for malaria and were allowed to treat non-severe cases of pneumonia (defined by respiratory rate) with amoxicillin. As a result of the study, the use of antimalarial drugs reduced significantly, but the use of antibiotics increased substantially (Yeboah-Antwi *et al.*, 2010). The design was not ideal, because the RDT they used could only detect *Pl. falciparum*, so a considerable amount of infections might have been missed.

The amount of counterfeit drugs is on a rise worldwide. It is estimated that 122,350 children in Africa died of a poor-quality treatment with these medications in 2013 alone. Also 17,000 samples of antibiotics, antimalarial and anti-tuberculosis drugs were investigated and 41% were found substandard. In addition, it is nearly impossible to withdraw drugs from the market that are not useful anymore. This should be considered when tackling malaria and other infectious diseases (Bate *et al.*, 2008; Newton *et al.*, 2014; ProMED-mail, 2015c; Schlagenhauf and Petersen, 2009).

The relationship between the parasite and its vector is unique because it influences the virulence of the disease. Usually, several blood passages will increase the virulence but then the transmission by a mosquito will reverse this effect. The molecular pathways for this reaction are still not clear (Spence *et al.*, 2015).

4.1.5 Traditional beliefs

Undoubtedly, many people keep to their traditional beliefs, be they educated or not. On one occasion during the survey, a witch doctor was present in the area and therefore participants were not available for their interview and blood sample collection. Generally people do not like to talk or share their knowledge about these beliefs. This is either due to being afraid of criticism or the malediction it may cause by sharing them with a stranger.

4.2 Materials and Methods humans

Since trypanosomiasis was investigated in humans and domestic animals, the same laboratory methods were used. This section will only highlight the additional methods used.

4.2.1 Study design humans

The study design chosen due to budget constraints is a cross-sectional survey in the same households where animal sampling and the household questionnaire took place. In a cross sectional survey only point prevalence can be estimated. It was decided to conduct a one stage cluster sampling with the cluster defined as one household. The calculation of the sample size is explained below. The ethical permission used is described in 1.9.5.

4.2.2 Sample size calculation humans

A census conducted between September 2012 and November 2012 was the basic sampling frame. The mean household size of the 1715 farmers in the sampling frame is 5.91 people, with a range of 0-27 people, compared to the mean household size of the whole study area population of 4.75.

For humans the sample size is based on malaria with an estimated prevalence of 50% (because this is a maximum of sample size). A more generous sample size would also increase the probability of detecting human sleeping sickness cases.

The resulting sample size can be seen in Table 4-1.

Table 4-1 Sample size calculation for cluster sampling in humans based on malaria

Species	number of individuals	number of households with data	percentage from all households	average number of people per HH	number of clusters	number of people to be sampled	level of confidence	precision	estimated prevalence	design effect (Shargie *et al.*, 2008)
humans	17656	3349	90.10%	5.27	97	512	95%	5%	50%	1.2

The human clusters to be sampled would be 49 in livestock and dog keeping households and 49 in households that do not own the investigated animal species to distribute it evenly. Taking into account the calculations for animal health and the household questionnaires, it was decided to sample humans in all livestock and dog keeping households (139) plus half of the amount of these clusters in non livestock and dog keeping households (70), which adds up ultimately to 210 households to be visited. It was expected to collect around 1107 human blood samples.

4.2.3 Sampling protocol humans

The sampling protocol was followed as described in 3.2.3. Each person in the household was sampled and interviewed provided a consent form was signed. In case people were illiterate, a finger print would be used as agreement. Parents would sign for their children under the age of 18. Copies of the paper consent forms are available at the University of Edinburgh.

4.2.4 Human health questionnaire

The questionnaires were conducted and recorded on tablets using droidSurvey software. Mainly two tablets with a bigger screen were used of the type Samsung Galaxy Tab 2, 3G and 8GB memory. Each person sampled in the household should have a matching questionnaire. The identification was based on an area code, household number, number of the person sampled per household, adult, age in years and sex of the person. Babies were recorded as baby and not adult and the age would be in months. Questions asked were in relation to questions on their sexual health, any drug abuse, any disease signs reported or seen, any medications taken and preventive behaviour. A detailed overview can be found in the annex.

Figure 4-4 Human health questionnaires were conducted away from others

Figure 4-4 shows a colleague recording the answers onto the tablet while other colleagues are sampling other persons in the household.

4.2.5 Laboratory analysis

The laboratory analyses for these studies were undertaken in two different places. The malaria diagnostic was based on a rapid diagnostic test (RDT) and used directly in the field. The laboratory analysis of sleeping sickness was conducted at the University of Edinburgh.

4.2.5.1 Taking the sample

For both the RDT for malaria and the test for trypanosomiasis, blood from a finger was used. The finger would be disinfected first and then pricked with a sharp single-use lancet so that enough blood would be provoked. The blood was then usually taken with a capillary tube but in cases where not much blood was produced, the finger would be held directly to the FTA card to spot on the blood. This was often the case with children or anemic people. The FTA cards would then be dried and stored at ambient temperature. An example for the blood sampling is shown in Figure 4-5.

Figure 4-5 Blood of a participant is brought onto a FTA card

4.2.5.2 DNA extraction in the lab

The DNA extraction was done the same way as with the animals samples as described in section 4.2.5.2. However, most human samples would not fully fill the circles on the FTA cards because the amount of blood taken from the finger was less. It was therefore decided to punch instead of five 3 mm holes only three 3 mm holes for the human samples. The quantities for the elution process when adding the chelex were reduced accordingly to 60 µl. The other steps like washing and drying were exactly the same.

4.2.5.3 Confirmation of DNA integrity

Since the results of trypanosomiasis testing may have far more impact in humans than in animals, it was necessary to confirm that the DNA was still in a good condition. To test for DNA integrity, the Cytochrome b PCR was used adapted from Kocher *et al.* and Muturi *et al.* (Kocher *et al.*, 1989; Muturi *et al.*, 2011). The focus is on the mitochondrial DNA. The mastermix preparation can be followed from Table 4-2. For each reaction, 25 µl of the master mix was added to 5 µl of the sample.

Table 4-2 Cytochrome b mastermix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Kocher *et al.*, 1989; Muturi *et al.*, 2011)

Cytochrome b PCR reagent	**Composition**	**Amount (in µl)**
Buffer	5x Mango Taq colored reaction buffer Bioline®	5
$MgCl_2$	50 mM	1
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer 1	10 mM (Cytb1)	1
Primer 2	10 mM (Cytb2)	1
Mango Taq	0.2 units/ µl	0.2
Water	DNA-se/RNA-se free MilliQ water	15.6
DNA template		1

The primers used for the Cytochrome b PCR are listed in Table 4-3.

Table 4-3 Primer sequence used for cytochrome b PCR (Muturi *et al.*, 2011)

Primer	Sequence (from 5'-3')
Cytb_1	CCA TCC AAC ATC TCA GCA TGA TGA AA
Cytb_2	GCC CCT CAG AAT GAT ATT TGT CCT CA

The Cytochrome b cycling conditions can be seen in Table 4-4.

Table 4-4 Cytochrome b cycling conditions

Cytochrome b cycling steps	temperature	time
1	95 °C	10min
2	94 °C	30 sec
3	52 °C	30 sec
4	72 °C	45 sec
5	Back to step 2 for 34 x	
6	72 °C	5 min
7	4 °C	forever

The expected band size is at 267 bp.

4.2.5.4 Diagnostic techniques trypanosomiasis

For the laboratory analysis of trypanosomiasis in humans two different PCR were used; TBR PCR and the multiplex SRA PLC PCR. More details will be given in the following sections.

4.2.5.4.1 TBR PCR

TBR PCR as described by Moser *et al.* is used to detect *Trypanozoon*, *T. simiae (Tsavo)* (Moser *et al.*, 1989). The protocol was adapted and the master mix prepared as described in Table 4-5; 25 µl of the master mix was added to 5 µl of the sample. For positive controls, *T. b. brucei* and *T. b. rhodesiense* had been previously passaged in laboratory mice as described by Welburn and Maudlin by Dr Ewan MacLeod (Welburn and Maudlin, 1987). DNA was obtained through use of a Qiagen DNeasy kit and stored at -20°C.

Initially, the TBR master mix was prepared using Biotaq Red DNA polymerase until its production was stopped.

Table 4-5 *T. brucei s.l.* PCR master mix preparation using Biotaq Red DNA polymerase for a total of 25 µl per sample (adapted from (Moser *et al.*, 1989)

TBR PCR reagent	Composition	Amount (in µl)
Buffer	670 mM Tris-HCI (pH 8.8 at 25°C), 160 mM $(NH_4)_2SO_4$, 100 mM KCI, 0.1% stabilizer	2.5
$MgCl_2$	50mM	0.75
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix (Tbr1&Tbr2)	10mM for each primer	1
Biotaq Red DNA polymerase	1 unit/ µl	1
Water	DNA-se/RNA-se free MilliQ water	14.55
DNA template		5

For the following samples, Mango Taq was used. One batch of samples was tested with the two different master mixes to ensure congruency of the results.

Table 4-6 *T. brucei s.l.* PCR master mix preparation using Mango Taq for a total of 25 µl per sample (adapted from (Moser *et al.*, 1989))

TBR PCR reagent	**Composition**	**Amount (in µl)**
Buffer	5x Mango Taq colored reaction buffer Bioline®	5
$MgCl_2$	50 mM	0.75
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix (Tbr1&Tbr2)	10mM for each primer	1
Mango Taq	0.2 units/ µl	0.5
Water	DNA-se/RNA-se free MilliQ water	12.55
DNA template		5

The primers used for TBR PCR are listed in Table 4-7.

Table 4-7 Primer sequence used for *T. brucei s.l.* PCR (Moser *et al.*, 1989)

primer	Sequence (from 5'-3')
Tbr1	CGA ATG AAT ATT AAA CAA TGC GCA GT
Tbr2	AGA ACC ATT TAT TAG CTT TGT TGC

The TBR cycle conditions can be seen in Table 4-8.

Table 4-8 *T. brucei s.l.* cycling conditions (adapted from (Moser *et al.*, 1989))

***T. brucei s.l.* cycling steps**	**temperature**	**time**
1	94 °C	3 min
2	94 °C	1 min
3	55 °C	1 min
4	72 °C	30 sec
5	Back to step 2 for 29 x	
6	72 °C	5 min
7	4 °C	forever

The expected band size is at 173 bp.

4.2.5.4.2 SRA PLC PCR

Those samples that were tested positively for *T. brucei s.l.*, were then processed using the multiplex SRA PLC PCR adapted from Picozzi *et al.* (Picozzi *et al.*, 2008). This PCR helps to differentiate *T. b. brucei* from *T. b. rhodesiense*. The same positive controls as for the TBR PCR were used.

The recipe for the preparation of the master mix is shown in Table 4-9.

Table 4-9 SRA PLC master mix preparation (adapted from (Picozzi *et al.*, 2008))

SRAPLC PCR reagent	**Composition**	**Amount (in µl)**
Buffer	Tris-Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$ (pH 8.7 at 20°C)	2.5
dNTPs	10 mM solution of dATP, dCTP, dGTP, dTTP	0.2
Primer mix	10 mM (SRA-F, SRA-R, PLC-F, PLC-R)	2
Hotstart Taq Plus	5 units/ µl	0.1
Water	DNA-se/RNA-se free MilliQ water	19.2
DNA template		1

The primers used for SRA PLC PCR are listed in Table 4-10.

Table 4-10 Primer sequences used for multiplex PCR (Picozzi *et al.*, 2008)

Primer	Sequence (from 5'-3')
SRA-F	GAA GAG CCC GTC AAG AAG GTT TG
SRA-R	TTT TGA GCC TTC CAC AAG CTT GGG
PLC-F	CGC TTT GTT GAG GAG CTG CAA GCA
PLC-R	TGC CAC CGC AAA GTC GTT ATT TCG

The SRA PLC cycle conditions can be seen in Table 4-11.

Table 4-11 Multiplex PCR cycle programming (Picozzi *et al.*, 2008)

SRAPLC cycling steps	temperature	time
1	95 °C	15 min
2	94 °C	30 sec
3	63 °C	90 sec
4	72 °C	70 sec
5	Back to step 2 for 29x and for 39x	
6	72 °C	10 min
7	4 °C	forever

A sample positive for *T. brucei rhodesiense* would show three bands in the agarose gel, one for the VSG gene (>1 Kb), one for the PLC gene (324 bp) and one for the SRA gene (669 bp). The presence of the SRA gene makes it uniquely identifiable.

4.2.5.5 Diagnostic technique malaria

In 2009, WHO-FIND and the CDC published a report on the test performance of rapid diagnostic tests (RDT) used for malaria (WHO, 2012b). They evaluated the different tests based in the laboratory. Criteria they were evaluating was detection at low parasite densities, low false positive rates, stable at tropical temperature and ease of use. The parasites detected would be primarily *Pl. falciparum* and *Pl. vivax*. The tests targeted the detection of either the HRP2 antigen, the pLDH antigen or both, where the latter were most successful. On the downside, test performance varied between lots.

The selected test Carestart Malaria HRP2/ pLDH Pan/Pf Combo has at low parasite levels a detection score for Pl. falciparum and Pl. vivax of 97.5 and 90 respectively. At high parasite levels the score is at 100 and 95 respectively. The false positive rate was at 3%. The test was performing well at different temperatures.

The test could then give seven different results as seen in Figure 4-6.

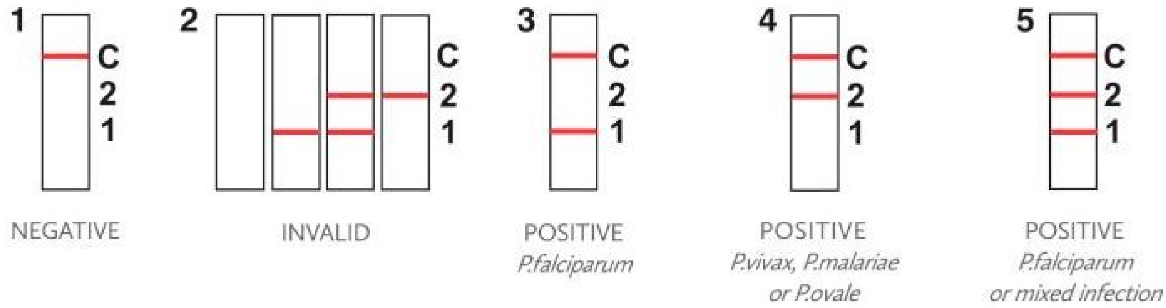


Figure 4-6 Interpretation of results CareStart Malaria Test

4.2.5.6 Sequencing

The company performing sequencing for selected samples was GATC. The Sanger sequencing method was applied.

4.3 Results

The data presented here are based on three different data sets; FTA card samples, malaria samples and human health questionnaire answers. In the chapter on the indicators for wellbeing, I will use the data derived from the wellbeing questionnaire which can differ slightly. These differences can have several reasons such as for example people refused to be sampled. They hence do not show up in the sample data but as part of the household data. Also to be noted, analysis relating to more than one data set can only use those data that are available for the required data sets. This means that if for example a question needs information from the FTA sample data as well as from the questionnaire, analysis can only be done for those people where both data sets exist. The aim was to have an FTA sample and a questionnaire from each person. Due to errors and other reasons, minor differences exist between the data sets.

In general, people possess exercise books where their personal health conditions, prescriptions and other data on their health are recorded every time they go to the health centre or see a doctor. So, the information does not stay in the clinic to that detail. This has the advantage that information is available even if the patient uses a different clinic or health centre.

This section also includes information from personal observation and communication in relation to the health status of the inhabitants of the area. Sometimes this is reinforced by literature.

A general observation made frequently in the area is that children have big bellies. When this was discussed with colleagues who are involved in human health, they said this may be due to a lack of protein or intestinal parasites. However, the main reason has not been investigated or is not known. Figure 4-7 shows a picture of children of whom some showed the protruded bellies.

Figure 4-7 Children were always happy to be in the focus of a camera; the girl on the left with the blue shirt shows the big belly that was observed often in children in the area

Plenty pictures were taken of children just because they enjoyed it so much to be in the focus of a camera and look at the picture afterwards.

4.3.1 Questionnaire related health status

The human health questionnaire and the household questionnaire provide data on health status and related risk factors. Data derived from the former are on an individual basis whereas the latter provides data for people living in one household.

The age and sex distribution of the respondents of the human health questionnaire can be seen in Figure 4-8. The age range was from 0-90 years.

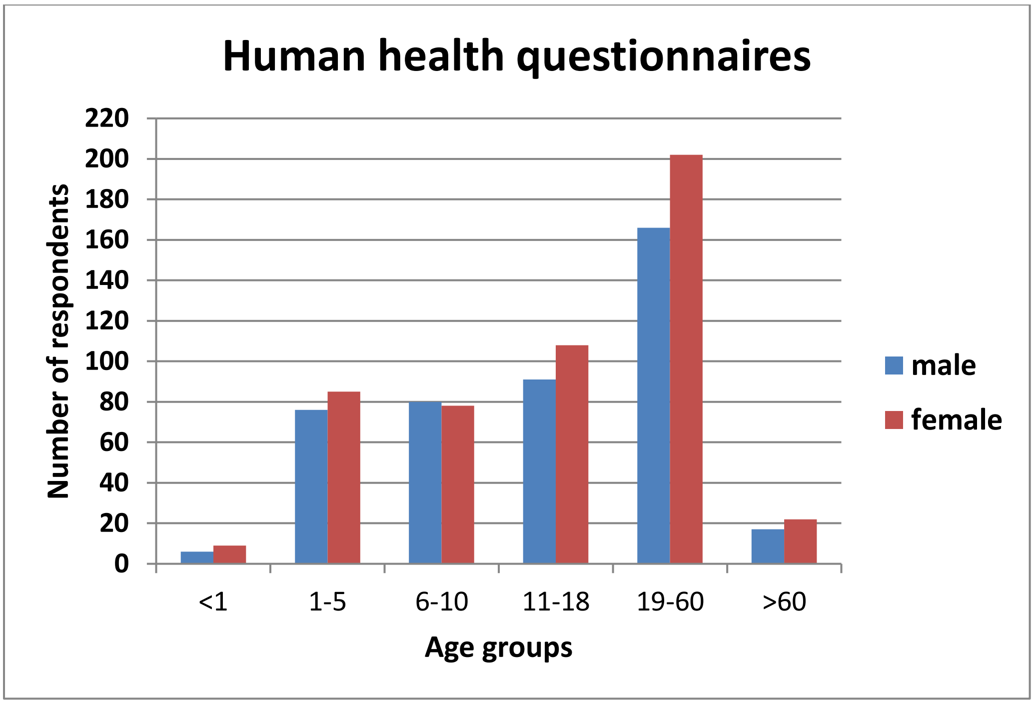


Figure 4-8 Human health questionnaires conducted (n=943)

The majority of respondents were children and teenager, and this corresponds with the population pyramid in low income countries. The sex distribution in teenager and adults is more in favour of women. For elderly people, this may be explained by a higher life expectancy of women. For teenager and adults, it could mean that men are often searching for job opportunities outside the area in bigger towns or cities.

4.3.1.1 Maternal and reproductive health

This section looks at the maternal and reproductive health of women. The discussion can provide an insight into the health services provided and the health status of women and their babies in the study area. Furthermore, it hints at the position of women in the society and the rights they have (Cook, 1993). Intimate partner violence hast been clearly linked to a reduction in use of contraceptive measure in women (Maxwell *et al.*, 2015). They would more often report to use condoms with their partner (Maxwell *et al.*, 2015).

From all female respondents being in or having gone through a reproductive age, 31% never gave birth (age range 13-84 years), 16% gave birth one to three times (age range 16-90 years), 13% four to six times (age range 16-69 years) and 14% seven to thirteen times (age range 30-85 years). The age given birth was between 13-29 years old. From the babies born, one to three were alive for 37% of women, four to six for 33% of women and seven to thirteen for 28% of women. The percentage of women that never had born a baby alive was 2%. For 6% the births were never attended, for 64% one to three births were attended, for 20% four to six births were attended and for 10% seven to thirteen births were attended. Of all women giving births, three quarters never had a miscarriage. For those that experienced one, 16% observed one, 6% observed two, 3% observed three and 0.5% observed even four miscarriages/ abortions. At the time of the survey, 4% of all women were pregnant, 12% were breast-feeding and 0.2% were both.

Looking at the birth control used, 24% of the women who answered the question did not use anything, 41% said they use injections without specifying the time, 19% used oral contraceptives, 6% used an injection valid for three months, 1.7 % used an injection valid for five years, 0.8% used the bilateral tubal ligation (BTL) and 7% used unspecified methods. The age range of birth control users was 16-61 years.

4.3.1.2 Symptoms and signs

Overall, 24% of respondents said that they were feeling sick, not well or showed any symptoms at the time of the interview. The relationship to malaria positivity was not significant ($p=0.66$). The reported signs and symptoms were headache, cough, stomach ache, body pain, fever, chest pain, dizziness, diarrhoea, sneezing, ear pain, anaemia, epilepsy, eye problems, joint pain, rash, tonsils, vomiting, swollen breasts, injuries and high blood pressure.

One additional measure taken was the body condition score divided into overweight, normal and underweight (skinny). The judgement was done by the interviewer. High weight was only observed in 0.2%, 4% of respondents were underweight and 95% were categorized as normal. The body condition score in relation to sex was statistically not significant.

There were many people not part of this survey who approached the team to say that they would need help because of their problem with epilepsy. They mostly claimed that the drugs did not work and very often were discriminated against by the society. The suspicion of a high prevalence of neurocysticercosis has been triggered by that. The affected persons received a small piece of paper which they could take to the clinic saying that this person needs to be checked for neurocysticercosis.

4.3.1.3 Prevention and treatments taken

When asked about medical treatments in the past 12 months, 60% of people said they had and 40% said they did not. Of those that received treatment, 97% went to the local health centre for it. There was no differentiation made between regular medication or medication for an acute disease. Drugs mentioned were mainly antimalarials, one mentioned an antibiotic and another one magnesium and folic acid. In fact, instead of providing the type or name of the drug, the majority of respondents would only be able to repeat the reason why they went for example headache. The only vaccines mentioned were against measles, polio, tetanus and yellow fever.

4.3.1.4 Drug (ab) use

In general, the consumption of alcohol or cannabis has a negative impact on health, e.g. oral hygiene of adolescents (Siziya *et al.*, 2011). Therefore the drug consumption was measured based on self-reported use. Alcohol, cannabis and tobacco consumption was asked in the categories ‘everyday’, ‘every week’, ‘every month’, ‘rarely’ and ‘never’.

Alcohol, tobacco and cannabis are consumed in declining order frequency. In general, drug use was not reported often and more in men than in women. Men sometimes use piecework to finance their alcohol consumption (Cole and Hoon, 2013).

The questionnaire found an alcohol use of never for 92%, rarely 3%, every month 0.4%, every week 1% and everyday 3%. The use of tobacco was never in 94%, rarely in 0.4%, every week in 0.1% and everyday in 6%. Marijuana, locally called *chamba*, was far less consumed with 99.6% reporting to never consume and 0.4% everyday.

The age range of alcohol consumers was from 17-77 years, of tobacco consumers 16-72 years and of marijuana consumers 22-44 years. Those that consumed marijuana also smoked tobacco.

4.3.2 Sleeping sickness prevalence

The population sampled for sleeping sickness prevalence can be seen in Figure 4-9. Younger age classes are in five year steps whereas adults above 50 years are in ten year steps.

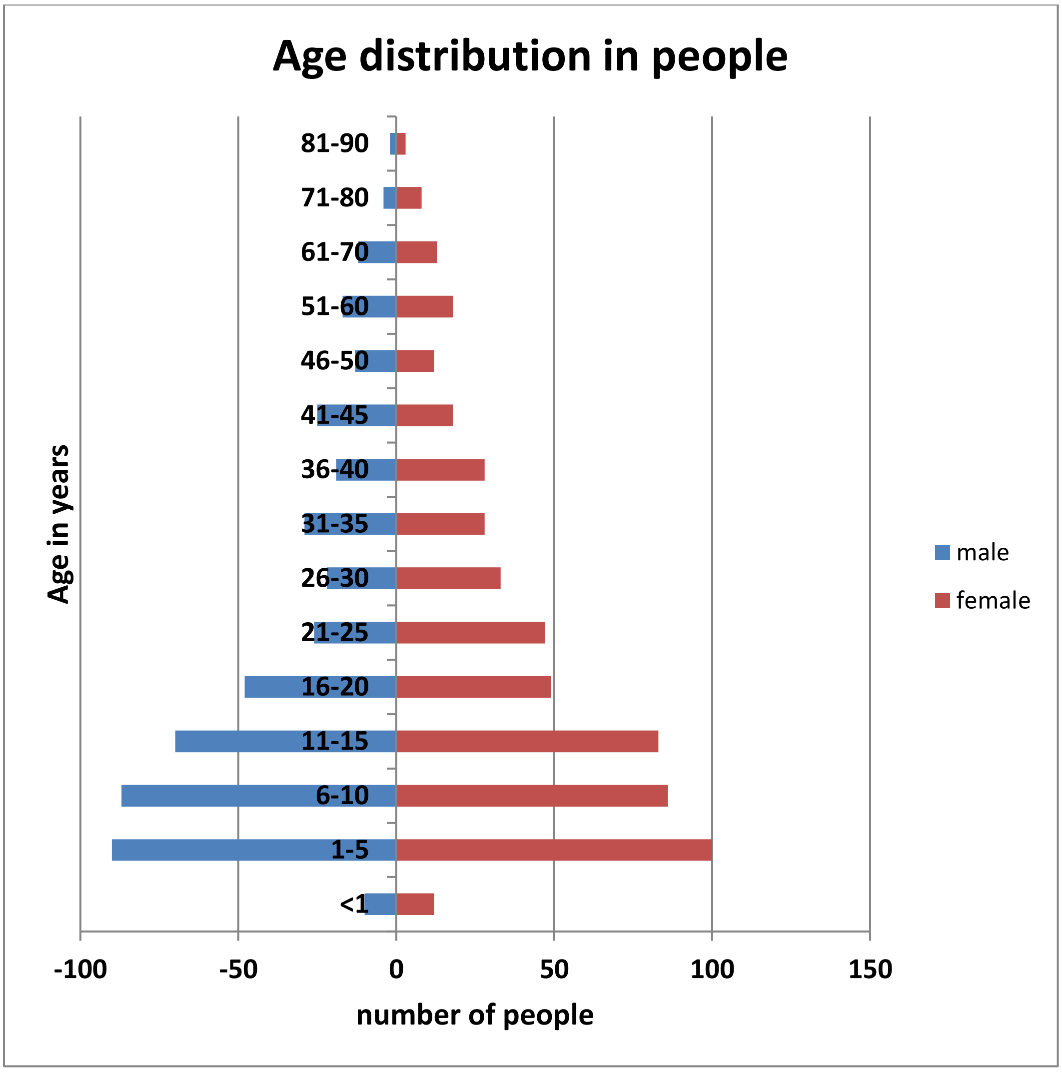


Figure 4-9 Human population tested for sleeping sickness (n=1012)

The geographical distribution of people sampled can be found in Figure 4-10.

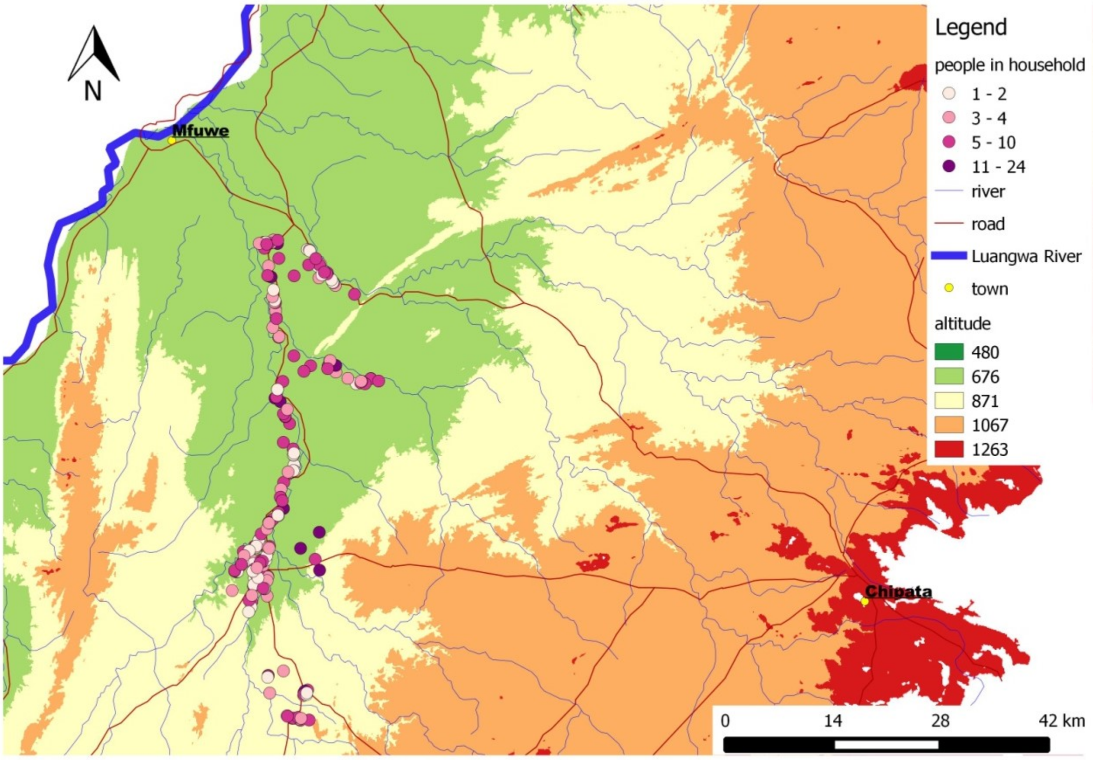


Figure 4-10 People tested for sleeping sickness

Eight human samples were positive once in a PCR protocol (TBR PCR), but this result did not repeat positive in another PCR protocol (SRA PLC PCR) or even the same one. Further identification of *T. b. brucei sensu lato* using multiplex SRA PLC PCR was overall unsuccessful. Additional research would be needed. I therefore considered all human samples for this study as negative for *Trypanozoon*.

4.3.3 Malaria prevalence

In total, 1005 people were tested for malaria using the CareStart Pf/ Pan RDT. The geographical distribution of the people tested and the household size can be seen in Figure 4-11.

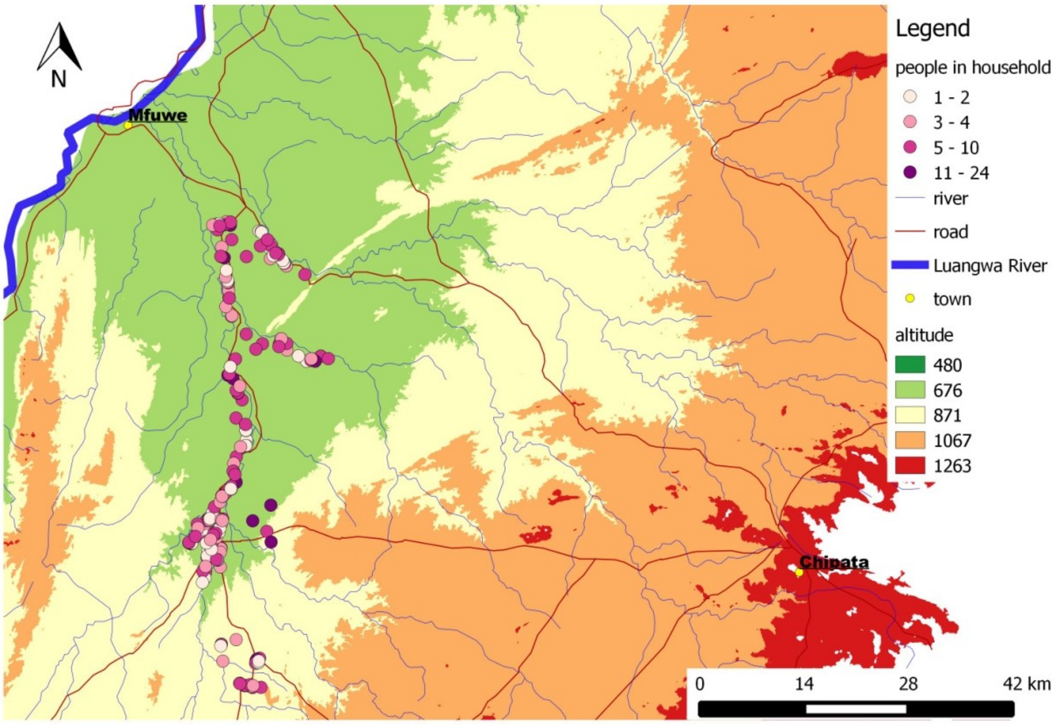


Figure 4-11 Distribution of people tested for malaria

The age groups for all people tested for malaria can be seen in Figure 4-12. The sex distribution is slightly shifted towards more women being tested, but that agrees with the general study population and cannot be interpreted in relation to malaria testing.

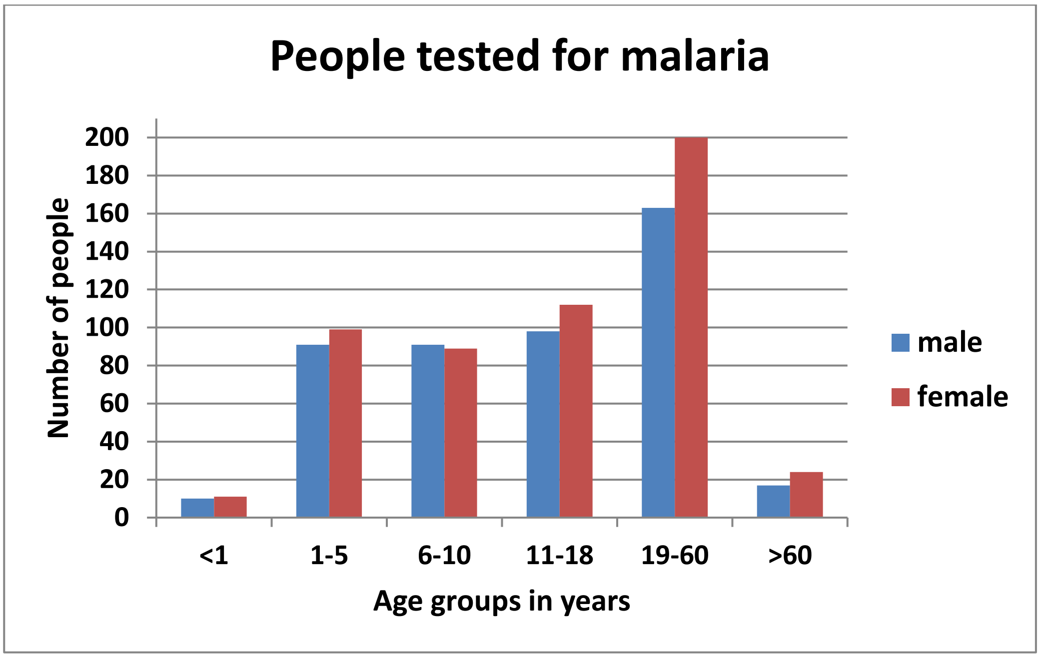


Figure 4-12 People tested for malaria by age and sex (n=1005)

From all samples tested, 85% were negative 15% were positive and 0.3% had an equivocal result. As indicated, the distribution of people testing positive per household can be seen in Figure 4-13. The red dots represent households with positive cases and the blue dots are households where no malaria was detected at the time of the survey. The map gives the impression that there are fewer cases in the north of the study area.

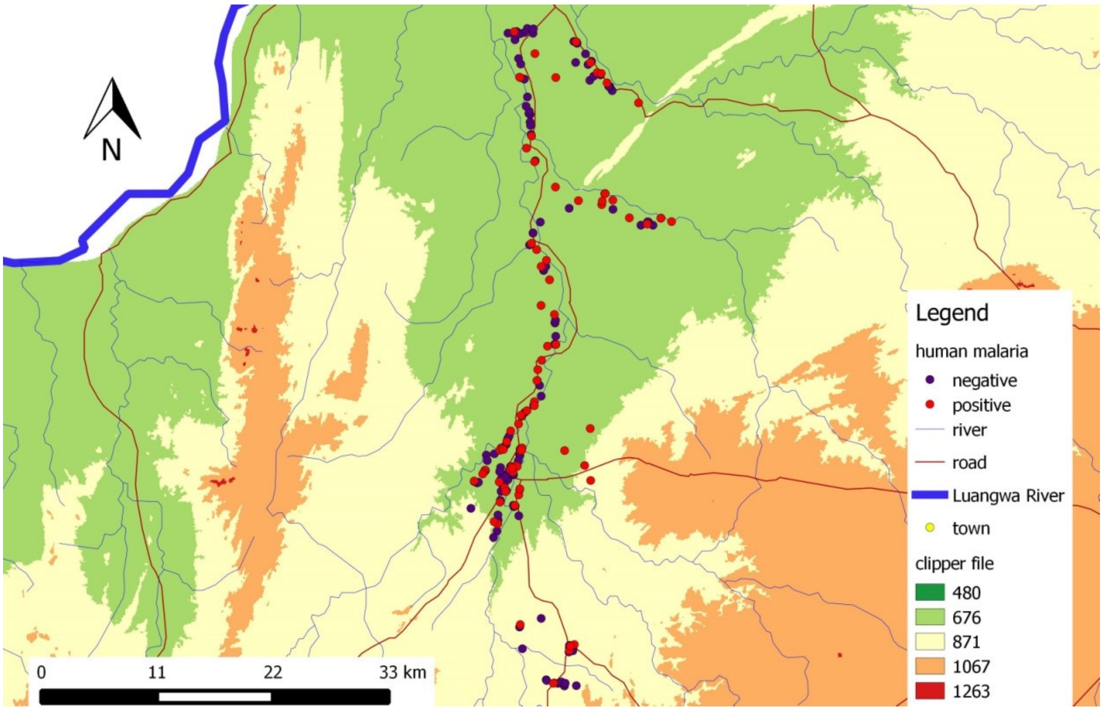


Figure 4-13 Households with malaria cases

Looking at the malaria burden per household, some households had more positive cases than others. These households are illustrated in Figure 4-14. Some of the households with a higher burden were also found with pigs that tested positive for cysticercosis (see 4.3.6.).

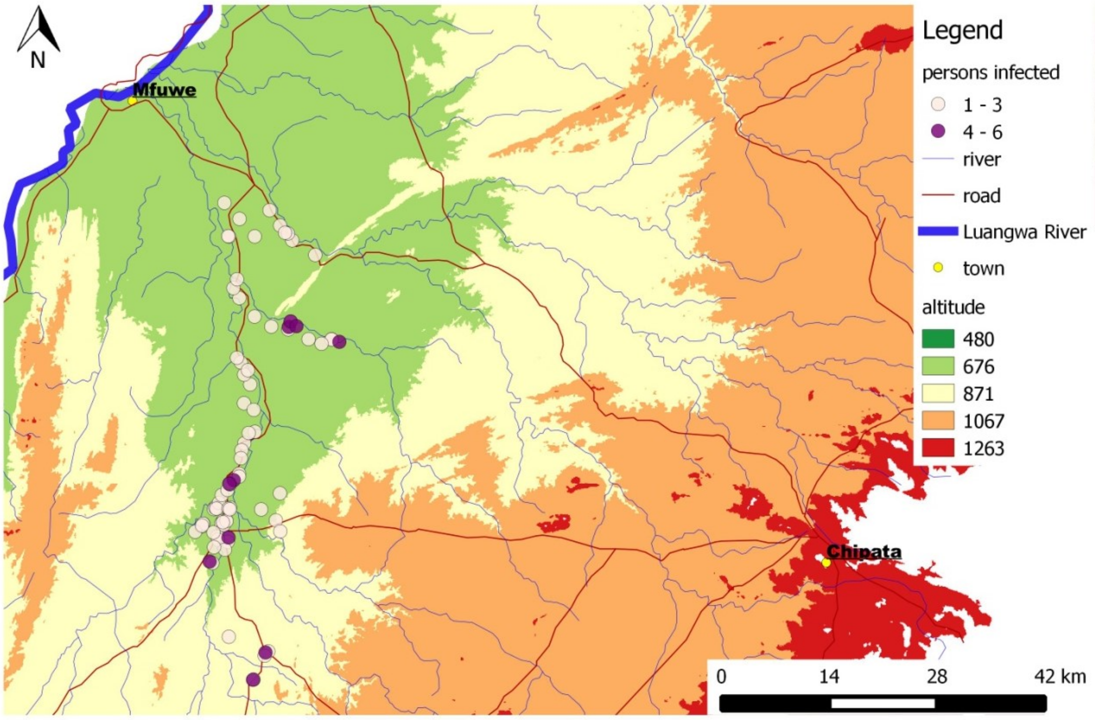


Figure 4-14 Number of people who tested positive for malaria per household

The positive samples could be divided into three different types; infection with *Pl. falciparum*, infection with *Pl. vivax, Pl. malariae* or *Pl. ovale* and a mixed infection with *Pl. falciparum* and at least one of the others. Unfortunately, the results were not registered to that detail from the beginning. Therefore 29% of positive malaria cases are unspecified (95%), 34% of positive cases were due to *Pl. falciparum*, 16% were caused by one of the other species and 21% had mixed infections. This makes a prevalence of 4.4% unspecified (95% CI 3.2-5.8), 5.2% *Pl. falciparum* (95% CI 3.9-6.7), 2.5% for one of the other species (95% CI 1.6-3.7) and 3.3% mixed infections (95% CI 2.3-4.6).

Figure 4-15 Another way of using a mosquito net to cover a puppy shed

There was no significant difference between men and women in malaria prevalence (p= 0.3344). The effect on age on malaria prevalence is shown in Figure 4-16 and Figure 4-17. The first chart shows how many cases there are per age group. It becomes visible that a quarter of the children from 1-5 years and more than a quarter of the children 6-10 years are infected with *Plasmodium* parasites. The error bars represent the margin of error in both graphs.

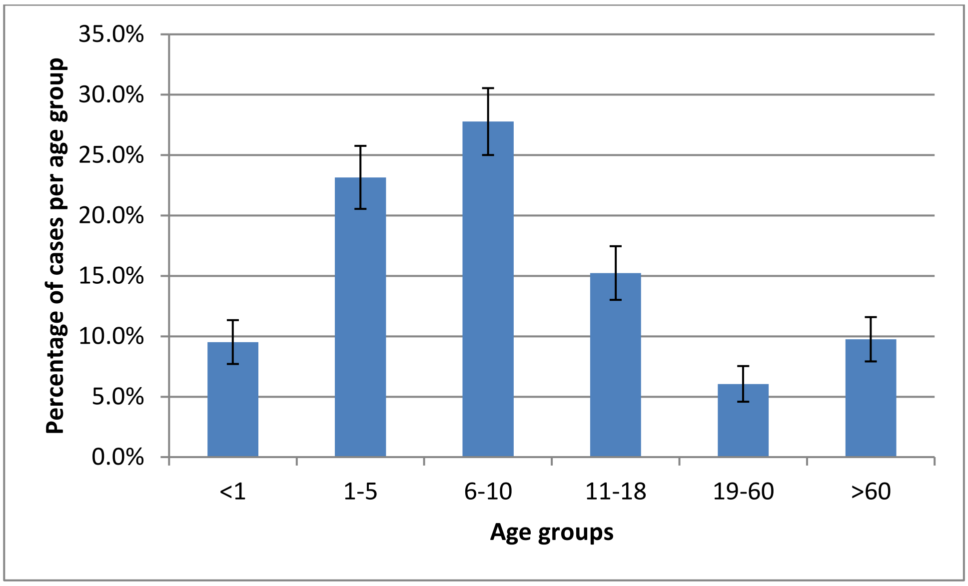


Figure 4-16 Prevalence within the age group

Now putting this in relation to other positive cases, Figure 4-17 shows clearly that more than 80% of the malaria positives are found in children and only to a small percentage in adults. Within adults, the semi-immunity that is usually developed for malaria may be the reason for the lower prevalence.

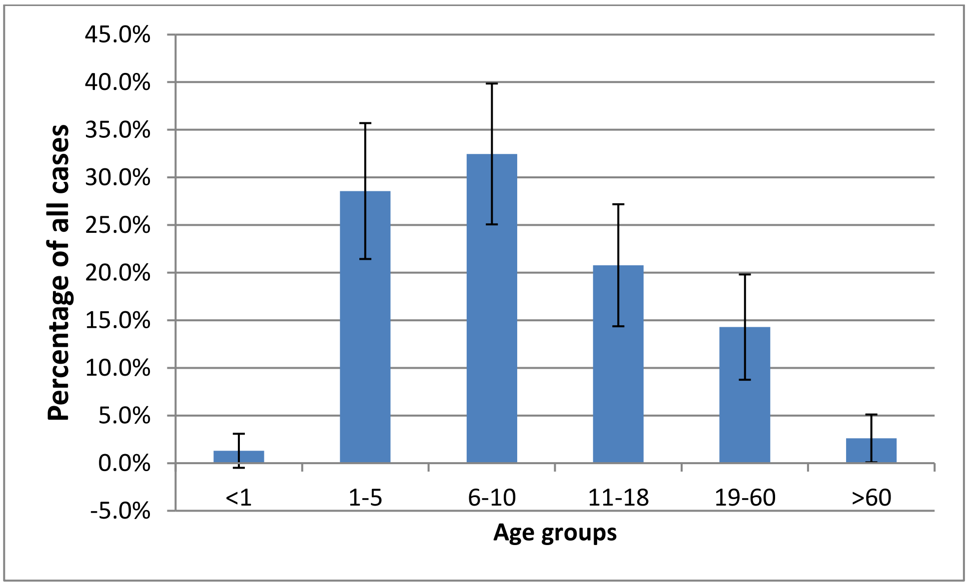


Figure 4-17 Distribution of age in all positive cases

From the literature we know that keeping animals is protective for malaria infection {Franco, 2014 #1686}{Jaleta, 2016 #1688}{Mayagaya, 2015 #1687}. So when it was tested for this study, there was a significant association (p= 0.0019).

4.3.4 Tick-borne disease prevalence

A subset of human samples was tested for tick-borne diseases. It is a very biased subset, because the people come only from households where animals had zoonotic TBDs and these people also showed signs or symptoms. Admittedly, testing humans for TBDs is not part of this thesis, but there was space left to test more samples during the last RLB. Out of 36 tested human samples, two were positive for *A. phagocytophilum*, two for *Ehrlichia/ Anaplasma* catch all and one for *B. caballi*.

4.4 Discussion

The reproductive health figures suggest that women do not have an easy life in the study area. The age of giving birth to a child at 13 years is too early and a risk for their health (Agbor *et al.*, 2017). The birth attendance rate has been increasing over the years though, so that is promising. One quarter of women giving birth had already previously miscarried, which suggests that even when the birth is attended by a qualified person it does not necessarily guarantee the survival of mother and child. Furthermore the limited influence women have on contraceptive issues and the ignorance by the health services of promoting the use of condoms is an interference with human rights.

Birth control injections and condoms at the national health centres are free in general. Condoms are given out when mothers go to the clinic with their children under five years, but they are not considered an option for contraception in women (personal communication Dr. Noreen Machila) (Gordon and Mwale, 2006). In rural areas, condom use is thought to be for HIV/ STI positive people only and that women do actually not have a say in their husbands using them (Gordon and Mwale, 2006). Condoms were also communicated as not safe because they have holes or do not function well. This was caused by the US President's Emergency Plan for AIDS Relief (PEPFAR) that funded a lot of national programmes on AIDS under the condition, that abstinence until marriage was promoted (Gordon and Mwale, 2006). Also, women need consent from their partners to be provided with an injection/ oral contraceptives. First-time applicants are usually asked to come with their partner for a counselling session (personal communication Dr. Noreen Machila). The only chance to get more information and maybe access would be again at the clinic check-ups for under five year children, since child spacing is encouraged to improve the health of the ones born. There are a lot of contraception-related misconceptions even in urban areas in Zambia and couples need to get more information and advice on the best method to use (Haddad *et al.*, 2013).

The low quality of reproductive health services in women has been noticed by government and there are projects to assess the best way of dealing with it (Ensor *et al.*, 2014).

The evaluation of the signs and symptoms reported together with the high occurrence of 25% of all people could not be related to malaria or sleeping sickness prevalence. This is a significant finding since it shows that other diseases are playing a role which might not have been considered yet. The mentioned cases of people having fits and the suspicion of neurocysticercosis are only one way to investigate into more detail the topic of human health. The symptoms and signs reported from the interviews are manifold and the low quality and quantity of health services and health staff in the area becomes obvious. The impression is there that going to the clinic with any ill-health condition will end in either a prescription of an antibiotic or antimalarial drug. Important to notice here as well is that people get treated with the same drugs or against the same cause (malaria) over and over again, regardless if they used self-medication before or had been prescribed the drug already. This may be due to a lack of trust to the patient, a lack of knowledge and means for alternative options or because drug resistances are really severe in this area. It is worthwhile to investigate the causes because that way the health system can be improved in general.

The consumption of alcohol, tobacco and cannabis were reported much lower than in comparable literature. But then the literature also mentions that this has happened before. The effect of drug use on the health status can therefore not be evaluated. From the figures provided, it seems that people consuming cannabis also consume tobacco and that if they are users, they are also frequent users. An association between tobacco use and mass media utilization was found in that men and women with mass media utilization such as radio, newspapers and TV, were less likely to use tobacco (Achia, 2015). This study showed a tobacco usage (smoking and smokeless use) in Zambia in women of 8.3% and in men of 12.9% (Achia, 2015). A review on tobacco smoking prevalence in sub-Saharan Africa between 2007-2014 included three studies from Zambia with respondents aged over 25 years (Brathwaite *et al.*, 2015). Smoking in rural areas was at 22.4% and in urban areas at 6.8%. More women smoked in rural areas (10.8%) than in urban areas (1.5%) (Brathwaite *et al.*, 2015). A national Zambian survey found male non-smokers of 76.3% and female non-smokers of 94.1%. Smoking was daily or irregularly (WHO, 2003). However, to have very distinct rural smoking estimates from two different cross-sectional studies during the same year or time period and similar area occur frequently (Brathwaite *et al.*, 2015). During the day, it was rare to observe anyone smoking or drinking alcohol at their homestead, but passing the dwellings in the car after sunset there was often the smell of marijuana in the air.

A similar phenomenon is seen with alcohol usage. A study population of patients receiving tuberculosis or HIV treatment were interviewed on their alcohol abuse. The prevalence of alcohol dependence was 27.2% in men and 3.9% in women (O'Connell *et al.*, 2013). A national survey conducted in 2003 found 9.5% male and 1.2% female infrequent heavy drinkers and 2.6% male and 0.5% female frequent drinkers (WHO, 2003). Very important to notice is that there is a strong link between alcohol use and sexual and physical violence towards women in Zambia. Women are more likely to be threatened and abused if their partner drinks alcohol (O'Connell *et al.*, 2013).

In a study looking at cannabis use in school adolescents in Zambia, the overall prevalence of self-reported ever-used cannabis was 37.2%. Factors significantly associated with marijuana use were history of having engaged in sexual intercourse, alcohol use, been bullied and supervised by parents during free time, the latter being protective (Siziya *et al.*, 2013).

Consistent with the results of this study, other authors also reported that the infections with *T. brucei rhodesiense* or the number of sleeping sickness cases have reduced in Zambia (Anderson *et al.*, 2015; Mwanakasale and Songolo, 2011). Possible reasons given are a change in land-use patterns and related destruction of tsetse habitat, an increase in regional migration, an increase in pesticide use on crops or an increased production of crops needing a high amount of pesticides such as cotton, less wildlife through increased hunting and poaching activities, under-detection because of an increased focus on HIV/ AIDS, malaria and tuberculosis and an possible increase in more asymptomatic or chronic infections (Anderson *et al.*, 2015; Mwanakasale and Songolo, 2011). The higher focus on malaria could be observed in the study area, but the size of the impact on sleeping sickness is not known.

The prevalence of malaria could include false positives since the CareStart RDT is known for this in the presence of HAT (Gillet *et al.*, 2013). However, the samples for HAT are considered negative and therefore should not have an effect on the prevalence of *Plasmodium* species.

The relatively high malaria prevalence in the area during cold season is not such a surprise if one looks at the picture in Figure 4-15. Mosquito nets often fulfil other purposes and are not used as mosquito protection. Either people do not take malaria serious, which was often the impression, or the effect of an ITN did not convince the population. It seems that sleeping below an ITN feels quite warm and stuffy. In former times, the bed nets were made of cotton which would provide more comfort than the usually used polyester nowadays (personal communications with the local population).

A. phagocytophilum has been found in dogs in Lusaka Province (Vlahakis *et al.*, 2017) and in humans in Morocco (Elhamiani Khatat *et al.*, 2016). An additional study found all mentioned microorganisms above in baboons and vervet monkeys from the Luangwa Valley in Zambia (Nakayima *et al.*, 2014). There are also case reports from the southern African region (Chitanga *et al.*, 2014). No reports of *B. caballi* in humans could be found. Further research needs to be conducted.

The results found for TBDs are unexpected and encourage a follow up study to investigate the role of ticks, TBDs and especially zoonotic TBDs in more depth. The role of wildlife needs to be further investigated looking at sleeping sickness and TBDs, as it seems that the abundance of wildlife has undergone drastic changes.

4.5 Conclusion

The people in the study area show a wide variety of health problems. Some are related to their reproductive health and others point at infections or chronic diseases. In general, the health system does not seem able to provide services for this wide variety, since the preventive measures and treatments received were very limited. Also the influence the health system has on human rights, especially the rights of women, is immense just by the restrictions on contraceptives and the conditions on their access and use. The positive usage of condoms is not promoted. Drug use seems to be surprisingly low compared to the literature. The fact that no sleeping sickness cases have been found could be due to restraints in diagnostic capacity or ongoing biological changes. The malaria prevalence was higher than expected per season and by national figures and the reasons should be further investigated. Similarly, the existence of TBDs never seen or reported before in humans in this area need to be looked at in more detail.

Chapter 5: Factors influencing well-being

This chapter focuses on the human wellbeing and poverty part of the study. Wellbeing is influenced by multiple factors, as mentioned in the general introduction. This chapter uses the results from the household questionnaire to evaluate the situation of wellbeing, with a particular emphasis on livestock-keeping, in the study area in Zambia during June till August 2013. An in-depth investigation into the dynamics of the socio-economic situation of households was not possible in a cross-sectional design. Key indicators that could provide an approximation are therefore used.

5.1 Introduction

The study area is a rural area in the Eastern Province of Zambia where agriculture plays the most important role in income generation. People living close to the national park can also work in the tourism sector or with wildlife. Parts of the area are very remote with virtually no access to markets or other infrastructure. This chapter outlines the infrastructure and some cultural characteristics and their possible impact on the livelihoods and well-being of the inhabitants of the Eastern Province. As often done in social science publications, the area-specific literature is intertwined with observations made and results obtained. The hypothetical basis for the themes covered is outlined in the general introduction chapter. The structure of this chapter is therefore slightly different than the others.

5.1.1 Aims of the study

The main interest here was to identify the problems farmers are struggling with in their everyday life and what is impeding their well-being. Therefore the aim of this chapter is to present the importance and the interplay of social, economic and environmental factors. These factors are culturally and geographically dependent, therefore the results obtained only apply to the area and time studied. The household questionnaires were conducted during June till August 2013.

5.2 Materials and Methods

The materials and methods provide details for the planning process such as the study design, the design of the questionnaire and the conditions adhered to during the implementation of the study in the field.

5.2.1 Study design

The exact sample size calculation for the animal health survey was done based on a census conducted in 2012. From this it was concluded that a sample of 139 households should be sufficient to detect trypanosomiasis with a prevalence close to the real prevalence. The exact calculation can be found in the chapter on animal health. However, for the evaluation of a possible impact of keeping livestock or/and dogs on household poverty, health status and wellbeing, it was necessary to add households that did not keep any mammals. Out of practicality and budget reasons the decision was taken to sample 50% more households, making a total of 210. One additional household was from the pilot study and some of its answers were used for analysis too.

The first version of the questionnaire was tested in interviews with colleagues from the field and adaptations were made. Then a pilot study with one household was conducted at the beginning. The questionnaire was taking too much time and the content was reduced accordingly.

Later on, each household was asked one household questionnaire, usually replied to by the household head and/or his wife. The average duration of each interview was 50 minutes. If the household kept domestic mammals, additional questions were asked and the interview could be longer. At the same time as the interview, blood samples were taken from all animals and people present in the household. In the cases where children were at school or animals were not able to be sampled, a maximum of two re-visits was made.

The day before the team would come to a household for sampling and interviews, a sensitization team visited the owners. The purpose and process of the survey was explained to them along with more information on selected diseases. This practice also enabled the confirmation of location of the household as well agreement to participate.

5.2.2 Questionnaire

The strategic development of the questionnaire was based on a literature review presented in the introduction chapter.

For the wording of the questionnaire, some questions were taken from related questionnaires with permission of the owner. The advantage here is that these questions had been validated already. Therefore credit should be given to Marie Ducrotoy, Noreen Machila, Vincenzo Lorusso, Ayo Majekodunmi and Alex Shaw, with inputs from work done by Jakob Zinsstag and Felix Roth.

The interview was recorded on a Samsung tablet 10.1 Note using the droidSurvey Software. The interview was conducted in an undisturbed place as can be seen in Figure 5-1.

Figure 5-1 For the household questionnaire, either the household head or his wife were interviewed

The applied questions can roughly be categorized into 14 different themes where one question could contribute to one or more themes. The themes are as follows; disease risk factors humans, disease risk factors animals, wellbeing, education/illiteracy, health status human, health status animals, poverty/socio-economics, animals traction, household demography, health services, veterinary services, livestock keeping, crop/ vegetable production and migration. Each of these categories may have small sub-categories of one question or more, e.g. decision power for wellbeing.

Regarding the type of questions, there are few open questions, most are single choice, some are multiple choice. There are also conditional questions where only a yes to the question if someone owns livestock or dogs is followed by how many of which species, sex and age etc.

The themes were inspired by the census questionnaire conducted. It contained a final open space for general comments and many would add valuable information. Communication with people in the area further enhanced the knowledge on culturally relevant factors influencing health, poverty or the wellbeing of the people in the study area.

The Annex contains the full version of the questionnaire.

5.2.3 Analysis of the answers

The type of questions differed between open text answers, conditional answers, single choice or multiple choice answers. Therefore the way the results were presented could be just narrative or simple quantitative percentages. In the case of crops grown and wildlife seen for example, the overall frequency of how many households had seen a particular species was presented. In most other cases such as the vegetation change, the mention of several answers per household would be considered as only one answer per household, since the questions were dependent on each other. So if a household was asked if they had noticed any vegetation change and they answered positively, only then they would be asked for the type of change.

5.3 General observations and perceptions

The information in the following sections is taken from area-specific literature, personal observations and communications.

5.3.1 Poverty in the study area

Data from 2008 report that 64% of Zambians live on less than 1USD per day compared to 21% of people from Malawi (Watkins, 2007). Poverty is strongly linked to the infrastructure of an area. The following are some examples that give an idea of the situation in the study area.

The sole source of electricity in Mambwe district is hydro electric power. It covers only the most populated areas around Mfuwe, the airport and Msoro. The supply is very unreliable with frequent power outages. Alternative energy sources are thermal, solar, fire wood and charcoal (ZAWA, 2011).

Available means of communication in Mambwe district are postal services, mobile communication, radio emissions, internet and television. However, in rural areas internet and television are rarely seen and postal services are only accessible in the Mfuwe area (ZAWA, 2011).

Bicycles are the most common means of transport. They are therefore affordable, accessible and widely used in the Eastern Province because there is a big bicycle producing company in Chipata (Eagle Bike Factory).

5.3.2 Livelihood strategies in the area

There is little opportunity of finding formal employment on a long-term basis in the area. Some people work for government bodies or private companies, e.g. energy supply or telecommunications. Tourism provides the major avenue for local people to earn some money on a fairly regular basis and without the need of a higher education. Often this is done through seasonal contract work (piece work). Other ways of generating money are the cultivation of cash crops such as cotton. Others grow non-timber products such as grasses and honey and sell them. A few people own small shops or businesses for the supply of basic requirements. Brewing beer made of maize and sorghum is a typical livelihood strategy especially amongst women. Animal husbandry has long been avoided in the study area, however over the last few years, some people have started keeping livestock and this contributes to their income (ZAWA, 2011). More detail on livestock farming is provided in 5.5.8.

5.4 Wellbeing in Zambia

Prescott-Allen compiled a set of indicators for the wellbeing of nations that is described in further detail in Chapter 1. For the grading of the indices, five categories are possible; good, fair, medium, poor and bad.

Zambia has a bad human wellbeing index (HWI) (Prescott-Allen, 2001). The health and population index for Zambia is also bad (Prescott-Allen, 2001). Any index related to wealth, be it household or national, is bad, except for food sufficiency it is poor (Prescott-Allen, 2001). Knowledge and culture are in the same categories (Prescott-Allen, 2001). Indices in relation to peace, order, crime, freedom and corruption are slightly better with poor to medium (Prescott-Allen, 2001). The equity indices and gender and wealth are poor to fair (Prescott-Allen, 2001).

The ecosystem wellbeing index (EWI) for Zambia is medium and the ecological footprint is good (Prescott-Allen, 2001). Looking again at the individual element indices, the land indices are all medium to fair (Prescott-Allen, 2001). The inland waters index is bad for Zambia (Prescott-Allen, 2001). The air indices are fair to good (Prescott-Allen, 2001). The species and genes indices are bad to poor, except for wild animal species which is fair (Prescott-Allen, 2001). The resource use indices are all fair to good, but the ones on fisheries are bad (Prescott-Allen, 2001).

5.4.1 Human wildlife conflict

The presence of wildlife does not only have beneficial effects on the study area. There are frequent conflicts happening between people and wildlife, either crop or property damage or through direct injury or death of people and domesticated livestock. Human wildlife conflict (HWC) is a well-known problem in the area and a number of methods have been implemented to try to reduce it. Birds and bush pigs usually only damage crops and are held back by rattling tins tied on ropes (ZAWA, 2011).

Figure 5-2 Fence around a field to keep wild animals away

More dangerous are monkeys, hippopotamuses and elephants that can raid crops but contact with them often results in human fatalities. People try to scare them away with drum beating and rattles. Elephants can also be sensitized and do not like to pass through a chilli fence. However, if these measures are not successful, monkeys or a hippopotamus may be killed. The most dangerous threat to people and animals are crocodiles, especially during the rainy season. If a crocodile causes a fatality, it will be hunted and killed (ZAWA, 2011).

5.5 Results from the questionnaire

The results from the questionnaires are intended to support the observations made above and will provide more details or figures.

Altogether, 211 questionnaires could be used for the analysis, including the one from the pilot study. From these households, 144 kept livestock or dogs and 67 households did not.

A problem encountered often was that not all animals or people were available or willing to be sampled. Animals would run away, or were not used to being handled by humans. Sometimes the owner would refuse to have his dogs sampled because he was scared they would lose their energy or aspiration to hunt. In humans, some people, especially children, might refuse, because they got scared and in some cases parents accepted their children's refusal. People recently tested positive for malaria were not sampled for that again.

Another common problem was that a woman would refuse to be questioned or for anybody to be sampled in the household without her husband being present. Fortunately, the sensitization conducted a day before would help to prevent this from happening too often.

All in all, the work was perceived well. The inhabitants of the area were happy that someone showed an interest in their problems and listened to them. Also the workers in the area were happy to share some of their experiences and through the funding of this project to be able to conduct more field work. However, some restraints were there because former projects had come and interviewed or sampled, but the results were never reported back. Of course this caused discontent among the participants.

A general impression perceived, not only by the author, was that female headed households are in a better shape. They may have fewer assets, but things were clean and tidy and the animals looked good. Some even had state of the art housing for their animals. Nonetheless female headed households are reportedly more deprived and have less food security (Freeman, 2008). One weakness of this study was not to differentiate between *de jure* and *de facto* female headed households. In the *de jure* households, women are widowed, divorced or separated, whereas in *de facto* households the women just live alone and the husband might have a job in the city and send some of the income home (Freeman, 2008).

Text box 5-1 Translations of answers in the questionnaire

Since there are at least two main languages in the study area, Kunda and Chewa, there were answers in the questionnaire, especially for diseases and wildlife species that were in different languages. Most answers were in English, but others could be in Kunda or Chewa. Chewa is very similar to Nyanja which is spoken in Lusaka and also in other parts of Southern Africa as Malawi and Zimbabwe. Kunda is only spoken by the Kunda people who originate from this area. Therefore the study area provides a blend of these different languages. I tried to translate these words with the help of my colleagues from the study area, but some of them have a double meaning because the word might exist in Kunda and Chewa meaning something completely different or because even within one language it can have two meanings. Additionally, disease names often describe a sign of the disease or its severity, but they cannot be directly related to one defined disease.

5.5.1 Demography

In the 211 households interviewed, five different tribes were recorded; Chewa, Kunda, Senga, Lenje and Ngoni. There were also few people who identified themselves as Nyanja, but then Nyanja is not really a tribe but a hybrid of local languages, also used in other parts of Africa. A map with the distribution of the different tribes can be seen in Figure 5-3. From observation and also confirmed by the map is the fact that Chewa are living further away from the main road. They often had big cattle herds and a lot of space but low infrastructure where they live.

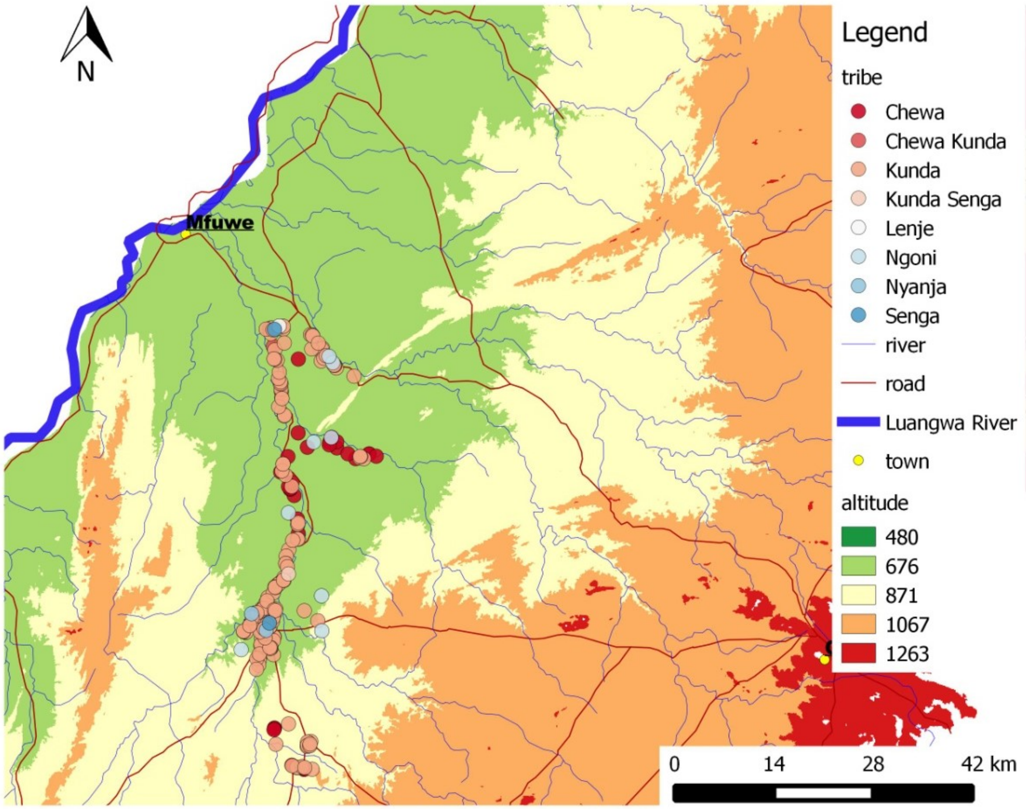


Figure 5-3 Tribe distribution in sampled households

The majority of households were Kunda (78%) followed by Chewa (15%) and Ngoni (4%). The other mixed households, Lenje and Senga represent 0.5-1% each. Looking at the sex of the household, 80% were headed by a male person. The Senga and Lenje households were female headed; all Ngoni and mixed households were male headed. Kunda households were in 78% managed by male persons and Chewa households by 87%. In 16% of the households, the household head was over 60 years old.

The average number of adult men living at a household was 1.2, the average number of adult women was 1.3 and children 3.5. This made an average household size of 6.1 people per household. Of the adult men, 77% were married and of the adult women 73%. In seven households, there were people above the age of 60 living completely alone; four were men and three women.

When looking at migration, 30% of households moved within the past 10 years. 60% of them moved within Mambwe district, 34% came from outside Mambwe district, but still within Eastern Province and 6% moved in from outside Eastern Province. The main reasons for moving are shown in a pie chart in Figure 5-4.

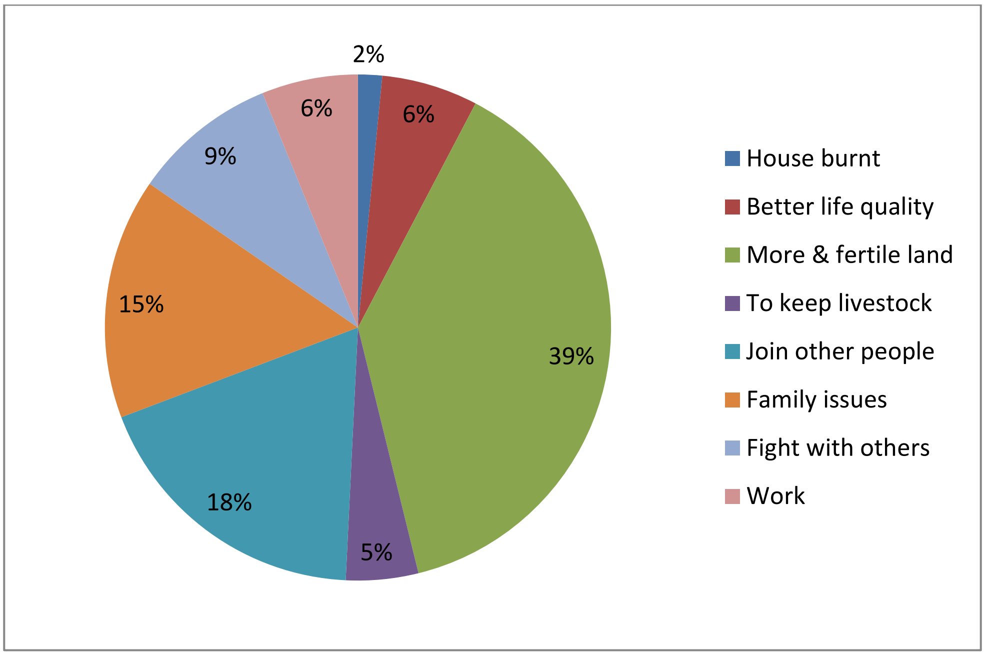


Figure 5-4 Push and pull factors for migration

Comments why it was better 'here' were; farming, family, less dust, better living standard, no floods, cheaper, community, more land, more fertile land, change of scene, clinic & mill close and less criminality. And to the contrary, comments what was worse 'here' included; food shortage, clinic and mill far, bad water quality, school far, soil infertility, same as before, more diseases, fight heights, wild animals, no business, too dry and too many snails in rainy season.

Because part of the area becomes inaccessible during the rainy season and due to wild animal raiding, 32% of the households move to their fields during the rainy season. The fields are between five minutes and 35 km away from their homes. Some are taking their animals and complete homestead and might have to rebuild everything when coming back (personal communication). That this type of seasonal migration has a negative health impact on humans, animals or both was the opinion of 64% of households moving.

5.5.2 Education

The question about highest education level reached per household had seven possible answers; none, basic, primary, lower secondary, upper secondary, college, other. The majority of households had the primary school as highest level (50%) followed by lower secondary (21%) and upper secondary school level (15%). College graduates were found in 3% of households.

Part of the human health questionnaire was to ask the interviewee if he was able to read and write. The three possible answers were yes, no and a little bit. Overall, 39% of the participants over 15 years said yes, 42% said no and 18% said a little bit. Looking at the sex distribution, 69% of people above 15 years not able to read or write were female and 31% male. This makes 52% of all female and 29% of all male persons above 15 years illiterate.

Looking at all age classes, 60% of interviewees never missed a day of school or work during the past 12 months. Nevertheless, 21% missed less than two weeks and 19% missed more than two weeks during the past year. From the people who never had missing days, 52% were female and 48% male. From all female people, 58%, and from all male people 62% had no missing days.

5.5.3 Risk factors human

This section investigates the exposure or probability of acquiring an infectious disease through risky behaviours or via the environment where people are living. Figure 5-5 gives a very good illustration of what is meant by that.

Figure 5-5 Dogs and chickens 'cleaning' the dishes- a possible source of sharing diseases

The figure shows the dirty dishes on the floor that have been used for cooking and eating and next to it there is a water storage container. One can see that the chickens and a little puppy are after the leftovers and licking the remains from the dishes. This may provide an increased risk for worm infections for example.

Provision of infrastructure alone does not improve the mortality. Education why a behaviour change is needed and what are the advantages and disadvantages improves significantly the health status. In the Luangwa Valley, education in conjunction with infrastructure is key to success (Sagan, 1987).

5.5.3.1 Water and sanitation

The water and sanitation situation in the study area is, depending on season and place of living, precarious. The majority of the population uses boreholes (56%). The remaining population uses wells (23%) or the river (20%). Less frequently reported sources of water were borehole and well together, a borehole with salty water or water from a tank. Figure 5-6 shows how to retrieve water from a dried river bed during dry season. The women are digging holes until the level of the water is reached and can be accessed. They then fill their containers with the water, trying not to have too much sediment in the container.

Figure 5-6 One way of getting water in the dry season is to dig holes into the dried river beds

Figure 5-7 is a picture of a man who is building a well for his household. The digging has to go quite deep depending on the ground water level and there is no technology used. The air in the hole becomes worse the deeper it is and the work can be quite strenuous. So apart from the risk of having an accident, the conditions of building a well are dangerous and sometimes life threatening.

Figure 5-7 Building a well is a dangerous task

Boreholes as in Figure 5-8 are the safest and preferred option to retrieve water in the study area. However, to build a borehole, more sophisticated technology is needed and therefore boreholes are usually only financed through development projects and not on the initiative of the surrounding households.

The advantage of a borehole is that the water access is not open and thus unlikely to get contaminated. On the ground, the water has to be pumped up, so spilling is rarely happening and animals are not free to use it. A disadvantage also observed in the study area may be, that the place where the borehole is installed has some geological peculiarity that changes the quality of water for consumption. The water in the study area was sometimes reported to taste salty, look orange or colour the teeth orange. It is likely that manganese plays a role in these areas.

Figure 5-8 A safer way of getting water is a borehole

People prefer using boreholes instead of wells wherever possible and this caused some wells to become unusable. In general, there is the complaint that the local community does not maintain their water sources such as wells and boreholes well enough and break downs are happening regularly. Due to lack of funds, the local authority cannot keep up with repairs (ZAWA, 2011)

Concerning sanitation and waste management it can be said that the presence of a pit latrine for each household is rather the exception but there are projects trying to promote their construction and use. Many times families share a pit latrine (ZAWA, 2011). When people were asked if they used a toilet regularly, 77% said yes.

Waste management is practically non-existent in the study area. Often garbage is just burnt or thrown away outside the living area. Only 18% of households reported ever receiving any hygiene training for human health. Another 23% said they never received any hygiene training at all.

On average, people had to walk 10 minutes to reach the next water sources. Nevertheless, some households needed up to 60 minutes. From all households, 74% said the water quality was good all year round, but at the same time 36% of the households said that there are times when water was scarce.

5.5.3.2 *Risk factors for selected diseases*

The risk of getting trypanosomiasis depends a lot on the abundance of the vector, the tsetse fly. Households were asked if they regularly saw tsetse flies on animals or people. The results are presented in Figure 5-9.

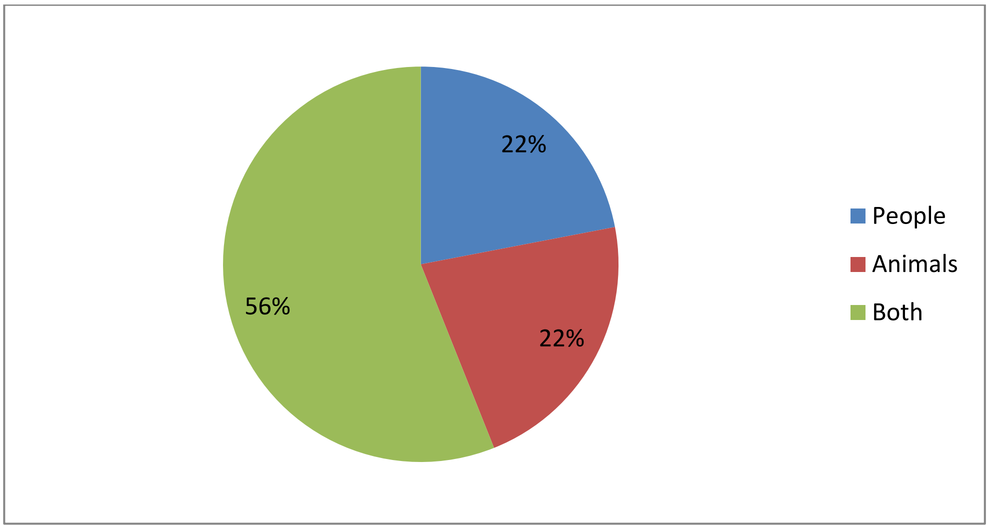


Figure 5-9 Number of households who observed tsetse flies

Furthermore the question was, were they seen usually, and if so when they would see them usually. The results are illustrated in Figure 5-10.

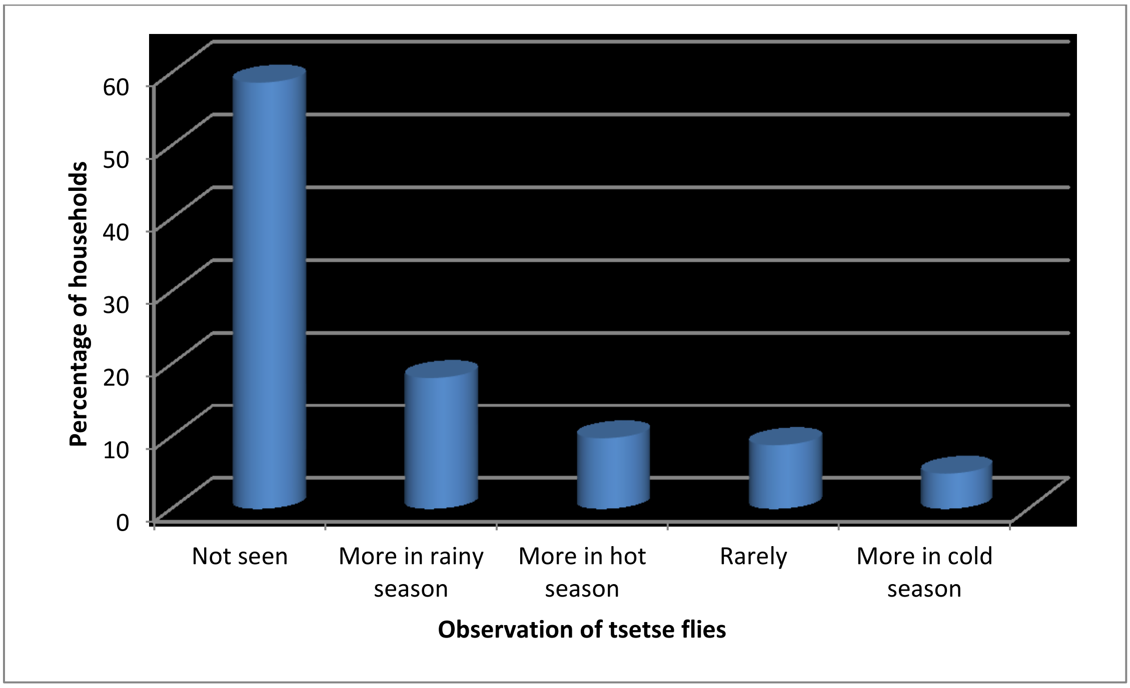


Figure 5-10 Observation of tsetse flies by households

The geographical distribution and abundance of tsetse was investigated by other colleagues within the DDDAC project.

Further to the vector burden, 18% of people reported finding ticks on their body and 31% never slept under an insecticide treated bed net.

Altogether 113 households answered the question whether anyone in their household had died since August 2012 (when the census started). There were 24 households who gave a confirmative answer and two of those passed away while either pregnant, during childbirth or six weeks after (the actual time of death was not asked for).

When asked if someone in the household had ever been bitten by a dog and died shortly afterwards, 125 households replied and nearly 10% said yes. Of these households, 83% kept dogs themselves.

Twenty three per cent of households reported a member being non responsive to malaria treatment. Their way of dealing with this was very diverse from going again to the health clinic, consulting a traditional healer, taking other drugs, praying etc.

Similarly, households were asked if they had experienced cases of fever during the past 12 months and only 25% said they never had cases. Thirteen per cent of households reported there was always someone with fever in the household.

There were no cases of diarrhoea in 35% of households over the previous 12 months Fifty nine per cent of households reported at least one case while 6% always had a case in the household. The result that a third of all households did not report a case of diarrhoea within the past 12 months might be due to underreporting within the household.

Due to high cysticercosis prevalence in neighbouring areas, people were asked if they knew someone having fits or acting bewitched in their area and if the number of people had increased within the last ten years. There were 54% of the households knowing someone and of these 40% said the number increased whereas 55% said it did not.

Another question in the household questionnaire was about the amount of food that was available to them and if it had changed as compared to five years ago. From altogether 211 answers, 53% said it was less, 12% said it was the same and 35% said it was more than five years ago. When interviewed about the existence of malnutrition and food deficiencies in the household, 11% gave a positive answer.

Looking at brucellosis risk, people were asked what they do with the aborted materials. From those who replied the question, 53% would bury them, 37% would do nothing and 2% would burn it, 2% use it for human consumption, 2% throw it into a pit or 2% give it to the dogs.

5.5.4 Risk factors for animals

When households were asked which diseases they would wish to control in their domestic animals, the answers were manifold. They used common disease names and local names as well as sometimes just the description of the clinical signs, species it occurred to and the season when it was most prevalent. Sometimes the same disease name, e.g. Newcastle disease (a poultry disease), is used for different animal species. This shows that it is difficult to distinguish if the farmer knows the disease and describes it or if he uses the word for a different disease in a different species with maybe similar symptoms or similar outcome. I therefore prefer to list the answers here to present the wide scope of descriptions rather than take any analytical or quantitative approach. However, I want to emphasise that these answers are still essential to consider in any process related to animal disease epidemiology, because they are observations directly from the field and can provide hints on what is going on at present. Some conditions are very obvious even to the occasional observer as seen in Figure 5-11. Other observations can be done only through regular involvement with the animals.

Figure 5-11 A bitch looking for leftovers to feed her puppies

Rabies and Newcastle disease are over proportionally mentioned, possibly because they are both quite dramatic diseases ending in the death of an animal or a person. It is therefore understandable that their names are easily remembered. But also here, Newcastle is mentioned in cattle for example, perhaps meaning a disease with a high death rate. Tsetse flies (*kamuzembe*), sleeping sickness, malaria in animals and trypanosomiasis are also often mentioned expressions and that shows that there have been considerable awareness campaigns to increase local knowledge and that the disease has a high burden in the area to be remembered well. Other parasites given are ectoparasites such as ticks and tick-borne diseases, especially East Coast fever (ECF), myasis and tabanid flies; and endoparasites such as worms especially in goats, but also other species.

Clinical signs mentioned by owners that were observed in their animals included abortions, diarrhoea, coughing, weakness, paralysis/ polio, emaciation, inflation of the stomach, *matekenya* (probably jiggers or myasis in animals), skin conditions such as ringworm, hairless patches, boils, lumps and scabies. Pig diseases are also considered a big issue, especially in the hot season, due to their high mortality, mentioned as African swine fever, swine flu, *chipumpu* and *chifine*. *Chipumpu* is a word used for a devastating and quickly fatal disease and was also mentioned for other species such as cattle (cough, mucus) and chickens (translated as Newcastle). *Chiwewe* (translated as rabies) is regarded a problem disease in dogs. Other diseases discussed were tuberculosis and foot and mouth disease. Seventy three per cent of households admitted that they did not know or were not sure to know any animal diseases, livestock disease or diseases in the particular species they own. Others said that goats do not have any disease which presents them as a robust species.

For 58% of households tick infestation presents a serious problem and they reported this to be mainly in rainy season (59%), but others said always (23%). It would affect all species. When asked if they found ticks in the environment of the animals, 53% would say yes or sometimes and 47% would say no. However, only 43% would use measures to control ticks in their animals.

Most of the households let their animals range freely (87%) and thus also mate freely with others (90%).

Text box 5-2 Role of dogs in the study area

Traditionally, dogs accompanied their owners to go hunting and therefore they need to be healthy, in good spirit and listening to the commands of their owner. Some owners would even refuse to let their dogs be sampled to not break their spirit and strength. However, the role of dogs in the study area does not show a clear picture.

On one hand, dogs were the most neglected species in the study area. Many were emaciated with a dull coat scared of their owners or any people around for that they would be kicked or beaten. It was very difficult to catch them for sampling, as they were so wary of the people around. Several dogs were running around with severe injuries, either from a conflict with another animal or from human violence. One had its nose off and another one bare testicles because someone had tried to castrate the dogs like a pig, realising it is not the same procedure. We helped one male dog who had had a very painful prolapsed penis for several weeks already, trying to reposition it. Many others had worms, maggots and fleas.

On the other hand, some owners would stand out as their dogs were very friendly, shaking their tails and letting us do any sampling we needed from them. They would listen to their owner and relax around the family, even the small children without any fear.

Interestingly enough, dogs have an economic value in the area. If a household has puppies or just bought one from someone, the dog will be locked inside overnight. This is not only to protect it from wild animal attacks, but also to prevent it from being stolen. This shows that dogs are a rare good, needed for hunting and security, but often the victims of mismanagement or wild animals.

5.5.5 Wellbeing

The subjective wellbeing of every person was investigated by only one question in the human health questionnaire. They were asked how satisfied they were with their life on a scale from 1-10 with ten being the highest value. Parents would answer for their babies and toddlers and judge their happiness by their behaviour. The results can be seen in Table 5-1. Interestingly, women's life satisfaction is more evenly distributed across all scores as compared to men.

Table 5-1 Life satisfaction in the study area

Scale	1-2	3-4	5-6	7-8	9-10	Total
Female	10	30	48	106	296	490
Male	1	22	50	70	265	408
Total	11	52	98	176	561	898

Figure 5-12 is a map where the life satisfaction, or average happiness, has been averaged per household and then plotted. The picture clearly shows that the majority of people seem to be satisfied with their lives. There are only some spots around Msoro and on the road towards Chipata that are less happy.

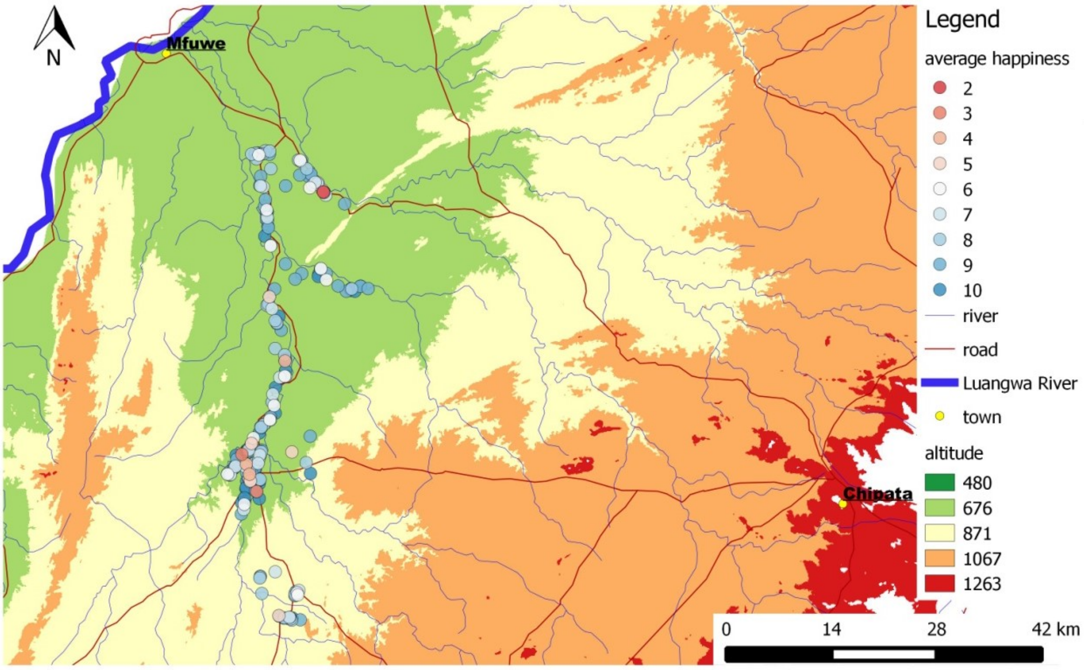


Figure 5-12 Average of overall life satisfaction per household

(scale 1 – 10 with 10 reflecting maximum satisfaction)

If happiness was visible it could have been in the household in Figure 5-13. The whole premise was kept nicely and the house was well decorated with the flowers. Few houses had drawings of wild animals or the family on their walls outside the hut.

Figure 5-13 Beautiful house with flowers on the roof

One question in the household questionnaire was if people thought that their lifestyle was better, the same or worse than five years ago. Forty six per cent said that it improved, 37% said it became worse and 17% said it remained equal.

Those people who had a worse or the same lifestyle as compared to five years ago were asked what they would change to improve it. Table 5-2 gives their answers (n=107). Interestingly, if added together, people's expectations from the government are high. A quarter of the households expect national help in one way or the other. Since the Zambian government also influences the education system, the health system and crop prices, its impact on the lifestyle of the valley people is immense. At the same time, it highlights the dependency of the people on the government, be it real to that extent or not. In the past, many development programmes have been implemented by or been conducted in cooperation with government officials. This might explain this strong reliance and high expectations.

Table 5-2 Changes that are needed to improve people's livelihoods and well-being

	Percentage of households
Improved farming	22.4
Government assist farming	11.2
Government support	10.7
Improved community cooperation	9.3
Better housing	5.4
Financial assistance	4.7
Better health system/ decreased mortality	3.7
Food	3.3
Business	2.8
Piece work/ stone breaking	2.8
Better crop prices	2.3
Fair distribution fertilizer	1.9
General support	1.9
Government support for old people	1.9
Fair allocation of usages by leaders	1.4
Improved market access	1.4
Bike/ vehicle	1.2
Increase farm size	1.2
Better education	1.2
Because of old age	0.9
Need a wife to help in the field	0.9
Practising witchcraft should stop	0.9
DDDAC provides help	0.9
Unknown	5.6
Total	**100.0**

5.5.5.1 Decision power

A related factor of wellbeing is the influence we have on our own life, the progress we want and the satisfaction we seek. In relation to that, when asked why they live where they live, the majority of 41% said that they chose the place on their own, 26% inherited the place, 17% were told by someone to live there and 16% had other reasons.

Kunda are matrilineal, but land is inherited through the male parent. There are two ways of acquiring land in the GMA by leasehold and customary law, where the latter plays the most important role (ZAWA, 2011).

5.5.6 Poverty and socio-economic considerations

One of the questions in the wellbeing questionnaire was what people would do if they had a little bit of money (50 Kwacha = 6 GBP, 2013) more per month. The amount of money selected is not enough to make big investments, but it is not so small to be considered useless. The frequency of answers is provided in Table 5-3. The idea of this question was to find out how poor people really are. If the investments listed would only represent rather unnecessary items, it would mean that basic needs are covered. However, a third of the households replied they would buy food or salt with this money. This shows that food variety is directly dependent on household income and thus can be a major cause of malnutrition.

Table 5-3 Ranking minor investments people would undertake with improved income

Minor investments	Percentage of households
Food/ salt	31.7
Business	14.7
Household goods	10.4
Better house/ land	9.2
Farming	9.2
Livestock	7.3
Personal hygiene	3.1
Education	2.6
Vet drugs	1.7
Dig a well	1.4
Clothes & shoes	1.1
Grinding maize	0.8
Bike/ transport	0.7
Build house for rent in Katete	0.5
Batteries for radio/ torch	0.5
Maintain wheelchair	0.5
Solar panel	0.5
Savings	0.2
Sleep comfortable	0.2
Taking children to hospital	0.2
Unknown	3.3
Total	**100.0**

Household goods and items for personal hygiene such as soap and lotions are a luxury too. Many households would like to save this money to invest it into better housing or improved crop and livestock farming through more land, drugs, fertilizer etc.

The questions on the conditions clothes, personal hygiene and dental health were not answered conclusively and cannot be analysed.

5.5.6.1 Goods and values

A common method used to assess the economic status of a household is by quantifying the assets it has. Comparing the results with other households in the same area or zone can support the understanding of wealth of the people there (Grandin, 1988).

As can be seen in Table 5-4, the main source of energy used for lighting is a torch with batteries. Out of experience during the fieldwork, it is known that the batteries are of low quality and do not keep long. Therefore alternatives such as candles and solar panels are used.

Table 5-4 Energy sources used for lighting

Energy source	Percentage of households
Battery/ torch	73.1
Solar	10.8
Candle	6.6
Paraffin	1.5
Electricity	1.4
None	1.4
Wood	1.3
Gas	0.0
Charcoal	0.0
Unknown	3.8
Total	**100.0**

The situation is different for energy sources used for cooking as in Table 5-5. Nearly everybody in the area used wood, and to a minor extent charcoal, to prepare their meals. The effect on the environment can be adumbrated.

Table 5-5 Energy sources used for cooking

Energy source	Percentage of Households
Wood	84.5
Charcoal	10.1
Battery/ torch	1.1
Electricity	0.6
Gas	0.5
Candle	0.2
Solar	0.2
Paraffin	0.0
Unknown	2.8
Total	**100.0**

Most of the year the climate would not make it necessary to use a heat source, but during the dry season it can become fresh at night. Therefore people use the wood or charcoal fire to keep warm as shown in Table 5-6. With over 27% of unknown sources, it would be necessary to ask again in more detail what that could be.

Table 5-6 Energy sources used for heating

Energy source	Percentage of households
Wood	49.9
Charcoal	12.5
Solar	5.1
Battery/ torch	3.6
Electricity	0.7
Gas	0.7
Candle	0.0
Paraffin	0.0
Unknown	27.5
Total	100.0

The household in Figure 5-14 is very modern because the roof is covered with an additional protection against rain and dust; it has solar panels on the roof and a satellite dish (purpose unknown) next to the house. Within the area, a household like this was unique.

Figure 5-14 Quite a modern way of housing with solar panels, a satellite dish and a canvas cover to make the roof waterproof

In various answers of the questionnaire it was mentioned that households would like to improve their housing situation. Figure 5-15 shows a brick house which is the highest standard to achieve in the area. When the house is finished it will have a corrugated iron sheet roof.

Figure 5-15 A fresh built brick house with roof and door still to come

Whatever the type of housing, be it a traditional mud house or a brick house, there will not be an internal bathroom or toilet. The common bathroom type used for taking a bath or for liquid excretions can be seen in Figure 5-16. The floor is usually covered with bricks or stones on one side to keep the feet clean when the soil becomes moist.

Figure 5-16 A typical bath or toilet hut

The range of farm sizes in the study area was from 0.5 lima to 250 hectares (1 lima = 0.25 hectares = 2500 m^2). The majority with 81% of the inhabitants owned a maize storage. The range of the size of maize storage was from 4 to 590 bags of generally 50 kg each.

An additional question was asking the availability and number of selected household assets in the households. Table 5-7 presents the results. It is important to note that more than half of all households possessed a bike and/or a radio.

Table 5-7 Ownership of selected household assets in the study area

Asset	Number of items owned (% of households)		
	None	One	Two or more
Mobile phone	36.5	36.1	27.4
Bicycle	29.3	51.9	18.8
Motor vehicle	97.6	1.4	1.0
Motor cycle	98.1	1.4	0.5
Solar panel	65.9	20.7	13.5
Television	79.0	19.0	2.0
Radio	39.2	51.7	9.1
Plough	85.5	9.7	4.8
Scotch cart	91.7	8.3	0.0
Sleigh	93.8	5.8	0.5
Sofa	89.8	4.9	5.4
Wheelbarrow	91.9	4.7	3.3
Fridge/freezer	99.0	1.0	0.0

Very few households in the area had the economic means to possess a motor vehicle, motor cycle or a fridge/freezer.

5.5.6.2 Resilience

The creation of and participation in clubs or cooperatives is a key to condense ideas and efforts for improvement in the area. These clubs or cooperatives can help to voice problems and improve the situation through collective activities. Development works at its best when mobilized citizens combine their efforts with government action (Evans, 1996). The type of club can vary. There are women's clubs, football clubs that are not only dealing with the sport, agricultural cooperatives for fertilizer and seeds, health clubs with volunteering activities and finally investment clubs. In the latter, members are collecting small amounts of money regularly from all members and once a month or when needed give this money to one person. In that situation, the increased lump sum can help a member to sort out a financial crisis in the household or make a bigger investment. It could be considered as a saving account in a bank. Members are morally obliged to pay their part and the distribution is fair so that everybody knows that they will receive the lump sum. In 1988, there were hardly any clubs or cooperatives existing in the area (Kalyocha G.C.K., 1988).

Female headed households in general have a lower resilience status, because they need to conduct tasks that are traditionally done by men (Cole and Hoon, 2013; Freeman, 2008). This may need a process of learning and training, but also makes acceptance within society difficult (FAO Regional Office for Africa, 2014; Freeman, 2008).

The question of whether there was an increase, decrease or no change in access to family/ community support networks as compared to five years ago was answered by 63% with an increase, 17% with no change and 20% with a decrease.

When the households were asked if they had compared to five years ago more, less or the same household savings to cope with illness or other problems, 55% said less, 10% said the same and 35% said more.

Text box 5-3 The blind woman

After realising that the number of people with fits was unexpectedly high, we decided to write a note to the hospital for every person we meet with fits about the suspicion of neurocysticercosis. In that way, their next visit to the health centre could help to provide correct diagnosis or treatment for the disease.

In one village we were introduced to a blind woman. She was single and a bout of seizures caused her to burn herself in the cooking fire. It is possible that both the blindness and the seizures are caused by neurocysticercosis.

There was a woman who was translating for us to talk to her, but other than that she seemed to be quite isolated in the community. Her hut was of poor quality and with low protection from environmental and other offences (part of it seen in the background of the picture). Usually this type of material is used only to build a ‘shower hut’.

Additionally, the woman was raped one night. Drunken men entered into her hut and one could hear her scream. The result of this night is the baby she holds in her arm. This woman made me think, what is the attitude of the other people in the village towards her? Are they excluding her from the society because of her ‘otherness’? Does she receive the help and support she needs? And finally, does her case say something about the overall support given to each other within the community?

5.5.7 Infrastructure of health system

The infrastructure of the health system was assessed in relation to its accessibility, the service provision and the satisfaction with the services. These three characteristics form the concept of UHC (WHO, 2017b).

Traditional healers were rarely mentioned, but through informal channels it was obvious that they played a role in the area. However, the magnitude of their role could not be assessed in this study.

5.5.7.1 Accessibility

The overwhelming number of households walk or use a bicycle to reach the nearest health centre as seen in Figure 5-17. The average time taken to reach it was more than one hour.

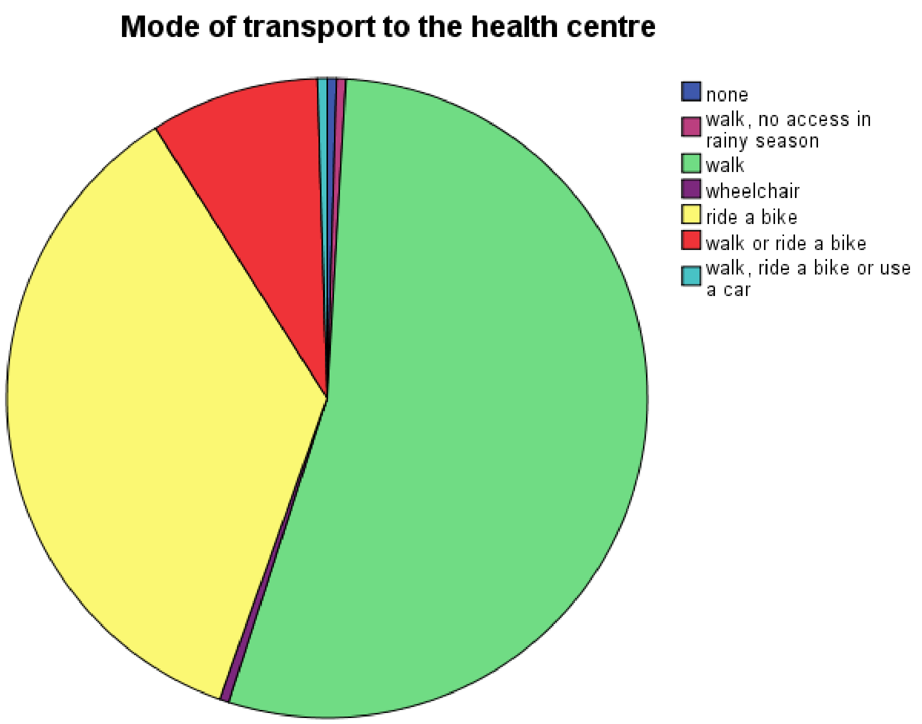


Figure 5-17 Mode of transport to the nearest health centre

5.5.7.2 Satisfaction

The satisfaction with the health services is quite high in the area since 71% of households confirmed this. The remaining 29% said there were long waiting times, lack of drugs, no ambulance, no consideration of elderly people, staff does not work on time and prescription of panadol for all illnesses. The active ingredient of panadol in Zambia is paracetamol, but most likely it is the only pain killer available in the area.

From the households that were not satisfied, 35% said they would not be willing to pay for a better accessible health service. One interestingly remarked that he would not be willing to pay because all government doctors sell hospital drugs privately. This brings up the problem of corruption. The others would pay if it was affordable, even for general services, some only for special services. One interviewee expressed his fear that there may be no money when sick.

5.5.7.3 Service provision

As already mentioned, the provision of hygiene training for humans was not very often reported. No other services were mentioned, although there seem to be maternal health services/ centres and voluntary HIV workers.

5.5.8 Livestock keeping

Most households in the area were keeping livestock because it is an important part of their income generation (84%), a food source (58%) and for traction purposes (19%). This was mainly looking at livestock but then dogs for example were mainly kept for protection.

The average number of animals kept per species per household was 2.5 cattle, 4.5 goats, 0.2 sheep, 2.6 pigs and 1.8 dogs.

Alternative income and food options are found close to towns or touristic areas. Therefore households keeping livestock are fewer there as seen in Figure 5-18. Traditionally, the Kunda people are hunters and therefore some households may also stick to their tradition instead of keeping livestock.

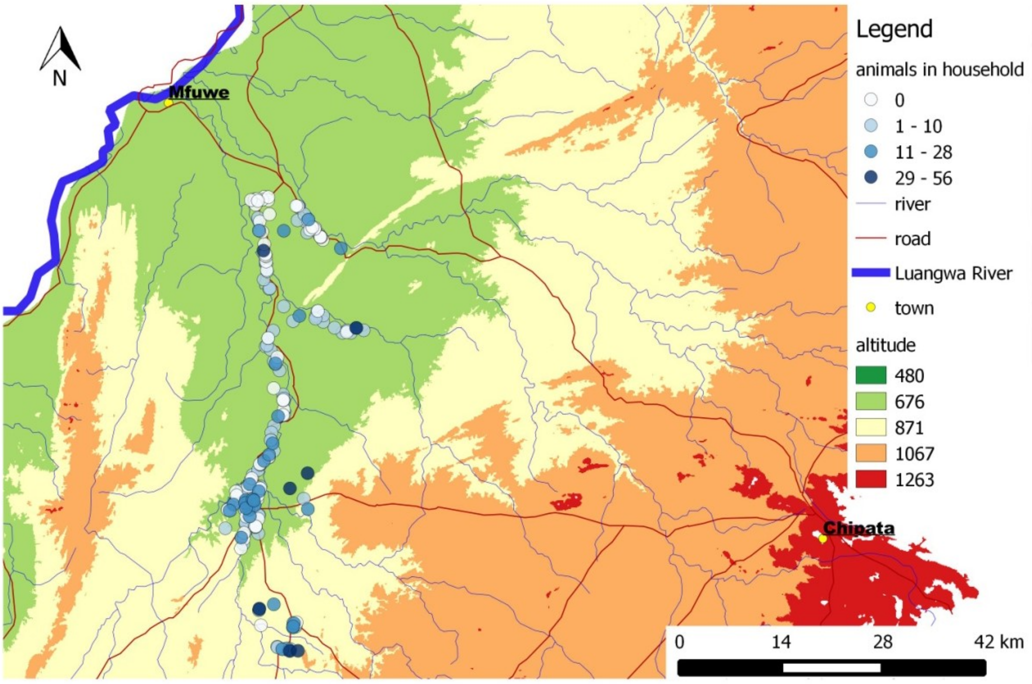


Figure 5-18 Map of animal keeping households compared to non-animal keeping households

Most animals would be owned by the household head (60%). Some households would say husband and wife (6%), other members of the family (7%) or the whole family (20%) owns the animals.

The frequency of the combinations of animal species kept in the different households can be seen in Table 5-8. It becomes obvious that most households keep goats and dogs.

Table 5-8 Distribution of animal species across households based on household questionnaire

Species owned					No of households	Percent-age of households
Goats	Cattle	Pigs	Dogs	Sheep		
X					24	**11.4**
			X		17	**8.1**
X			X		16	**7.6**
		X			16	**7.6**
X		X			12	**5.7**
X		X	X		10	**4.7**
	X		X		10	**4.7**
X	X		X		5	**2.4**
	X				5	**2.4**
	X	X	X		5	**2.4**
		X	X		5	**2.4**
X	X	X			4	**1.9**
X	X				3	**1.4**
X	X	X	X		3	**1.4**
	X	X			2	**0.9**
X	X			X	1	**0.5**
	X			X	1	**0.5**
				X	1	**0.5**
				X	1	**0.5**
					70	**33.2**
					211	**100**

The feed situation in the study area during the past five years seems good with 87% of households having enough for their animals. Only 13% of households said that, in some years or always, they did not have enough to feed their animals.

5.5.8.1 Livestock population dynamics

The population dynamics of the different animal species provide some insight into the health status of the animals but also into the economic impact that is implied by keeping one species or the other. To be able to calculate these dynamics, the households were asked in the household questionnaire which species they had and how many of them they owned. The calculation is shown in Table 5-9. The owner would then be asked how many of these animals had died or were born during the past 12 months. In smallholder livestock systems it is quite common to give animals for a gift, sale or in custodial care. Together these exits are called the offtake. In addition to that, they can receive animals as a gift, purchase or in custodial care. Those animals are the intake. Through these given figures on exits and entries, the number of animals present 12 months ago can be calculated. Comparing these to the present figures a number of conclusions can be drawn.

Table 5-9 Productivity calculations for animals

	Goats	Cattle	Pigs	Dogs	Sheep
Number at time of interview	576	316	328	233	26
Births	268	49	201	90	4
Intake	43	50	25	22	1
Deaths	49	23	84	29	0
Offtake	122	17	35	16	2
Number one year ago	436	257	221	166	23
% deaths	11.2	8.9	38.0	17.5	0.0
% rate of growth	32.1	23.0	48.4	40.4	13.0
% natural rate of growth	50.2	10.1	52.9	36.7	17.4
% offtake	28.0	6.6	15.8	9.6	8.7
% intake	9.9	19.5	11.3	13.3	4.3
% net offtake	18.1	-12.8	4.5	-3.6	4.3

The rate of growth is determined by calculating the difference between the number at the time of the interview and the number one year earlier dividing it by the number one year earlier. It would then represent the growth in the population. The natural rate of growth is the difference between births and deaths. The percentage of the offtake and intake represents the dynamics of trade in the population and the net offtake identifies the trend if more animals were given or received. A positive net offtake means more animals are given away and a negative rate means more are received.

The sheep are so few that it is difficult to use these numbers realistically. The death rate for pigs at 38% is very high and probably due to African swine fever outbreaks. The death rate in dogs is also quite high as compared with the other species. Cattle and dogs are more often received than given away. The natural rate of growth is lowest in cattle.

The calving rate in cattle can be also calculated. Taking female animals from the age of 12 months who are pregnant or have a calf plus the ones aged more than 48 months, 29% of the herd are cows. That implies a calving rate of 59% based on the average number of cows present during the year. From his surveys in high and low tsetse challenge locations in Zambia's Eastern Province, Doran obtained a calving rate of 44% in Petauke (high tsetse challenge) 50% in Katete (low tsetse challenge) 37% in Chipangali (high tsetse challenge) and 42% in Feni (low tsetse challenge) (Doran, 2000).

One of the factors influencing the population dynamics is the lack of market outlets for animals and animal products. There are no significant market places and therefore 62% of people are not selling their animals or animal products. Others give their animals to anybody, the church/ mission, teachers, traders from Katete or Chipata and the clinic. However, as compared to five years ago, 64% said that the livestock market had improved and 33% said it became worse.

Only 12% produce and/or consume milk and milk products from their own animals. From those, 65% said that they boiled or treated the milk before consumption.

Reports of abortions, stillbirths or weak-born offspring during the past 12 months were noted in 37% of the households keeping animals.

5.5.9 Animal traction

Animal traction plays an important economic role in many developing countries (Randolph *et al.*, 2007). From livestock keeping households, 29% owned animals for traction. A farmer owning traction animals can also hire them out and earn some cash through this, but only 55% of them would hire out their animals for traction for, on average, 30 days a year. From these households, 69% had draft cattle that they could not use during the last six months because they were sick, on average for 8 days. Only 9% of households reported that they had used someone's cattle for ploughing or other draft work.

Farmers start with young heifers or bulls and train them to walk together with a harness. This can be seen in Figure 5-19.

Figure 5-19 To get the cattle used to walking and working together their owners let them walk around with the harness

(© J.Kuleszo)

When cattle are already more experienced and regularly used for traction it looks like in Figure 5-20.

Figure 5-20 One way of using animal traction in the area

In our study, nearly 3% (4/139) of female cattle were used for traction. The majority were bulls, with 54% (86/158) of male cattle used to transport heavy load or to plough fields. The age of these working animals ranged from 6-264 months. Even one goat was mentioned as being used for traction.

5.5.10 Infrastructure of veterinary services

The veterinary services were similarly evaluated as the human health services, according to the UHC concept of accessibility, satisfaction and service provision (WHO, 2017b).

Theoretically, everyone has to pay a fee for a licence for keeping a dog. In Lusaka this costs 30 ZMW, but it may vary from council to council. It is not clear if the licence fee is a flat rate for dog ownership or if it increases with every additional dog. However, the law is not reinforced. There is also a maximum number of dogs a person/ household is allowed to keep. Also how to register and report a dog's death is not clear (personal communication Dr. M. Simuunza).

5.5.10.1 Accessibility

From those households that kept animals, 77% reported that they did not have any veterinary services in close proximity and 2% mentioned only irregular visits. The distance to the next veterinary facility was given as between 1-100 km.

Only 33% of livestock keepers had constant access to veterinary drugs, 6% only sometimes or for some drugs and 61% had never any access. Possible locations where they would get the drugs are Katete, Chipata, Jumbe, Msoro, local shops, Kasamanda, Kamulele veterinary office, Mambwe, from one of the tsetse control assistants, mobile traders and Sinda (a town along the great east road).

5.5.10.2 Satisfaction

During the study, many complaints about the veterinary services were expressed. When livestock keepers were asked what improvements they would like to see to current animal health care provision, the answers were manifold; dip tanks, regular veterinary visits, vaccination, availability of veterinary drugs, animal production and health training, more feed, general support, how to keep bigger animals, how to improve animal keeping and water dams for the animals. One mentioned that the veterinary drugs had to be improved because a lot of animals die of the drugs provided so far. Often these services were expected to be provided by the project, the government and in general for free or for a cheap price. The vaccination of dogs was mentioned several times. Furthermore some farmers were asking for improved services for animal species they did not themselves own.

Because of the very limited availability of veterinary services, 72% of livestock keepers were willing to pay for better accessible (private) veterinary services. An additional 10% agreed with restrictions such as affordability and for special services. Altogether 4% said they were not able to afford that.

5.5.10.3 Service provision

Treatments given for acute diseases were Berenil (diminazene aceturate), Samorin (isometamidium chloride) and oxytetracycline. One farmer said he keeps his pigs in quarantine when he hears of an outbreak. Another farmer said that he mixes salt with battery powder to cure his animals. In addition, one mentioned giving his pigs Fansidar (sulfadoxine and pyrimethamine), an anti-malarial drug. However, most notably, all in all, 78% of households had not given any treatments to their animals.

Investigating the use of preventive measures and vaccines, 10% of households used a dewormer. Given the opportunity to mention other measures, they mentioned again Berenil, Samorin and oxytetracycline. In addition single households said that they apply home remedies (crushed pumpkin seeds), spraying, dipping, salts, report to wildlife conservation and vaccinations. One household said they are sharing their vaccinations with the animals to protect themselves.

Only 19% of livestock keepers had received training on hygiene and/or animal production methods in the past.

The responsibility of the government in animal health care is overlapping with the improvements householders would like to see in general. Answers mentioned water dams, improved veterinary services, availability of drugs, training on animal production methods, distribution of fertilizer not just to the friends of the officials responsible, create more clubs, give them livestock, vaccination campaigns, dip tanks, eradication of tsetse flies and soft loans. Interestingly, a few households said there is nothing.

The responsibility of the private sector in animal health care was seen in the stock kept and the sale of affordable and non-expired drugs, provision of special services. However, other points made by respondents were that the private sector could not help because they are business people, the farmers had no money for private services, training on improved livestock keeping, there should be no counterfeit drugs, it would be useful to train a local person in the community, information and advice should be provided, increase in women's groups and licences to sell drugs. The need for the provision of drugs was by far the most frequent answer.

The responsibility of the farmer is perceived as being to take care of the animals, to provide feed and keep the kraal clean, to monitor the wellbeing of the animals and report any problems, to provide money to buy drugs, to improve housing of the animals (especially that animals are not soaked during rainy season), to sell and eat them, to keep animals healthy, job creation for others, to deworm chicken and to restrain/quarantine animals when needed.

5.5.11 Crop and vegetable production

Most of the households grow their own crops. The crops have two purposes; they are either produced for consumption or as cash crop. Cotton is a typical cash crop in the area and grown by the majority of households (83%). Many bags are produced each season Figure 5-21.

Figure 5-21 Cotton bags waiting to be picked up by the company

The crops commonly grown in the area are listed in Table 5-10. Maize can be either the traditional one or a hybrid one. The former is used for consumption the latter for sale since the local people do not like the taste of hybrid maize (Kalyocha G.C.K., 1988).

Table 5-10 Crops commonly grown in the area

Crop	No of households growing crop (as a % of all households)	Absolute no of households
maize	97.2	199
groundnuts	82.0	173
sunflower	16.6	35
cow peas	10.4	22
rice	7.6	16
cassava	3.3	7
sweet potatoes	3.3	7
nyemba beans	3.3	7
rape	2.4	5
vegetables/ gardening	1.4	3
bananas	1.4	3
millet	0.9	2
pumpkins	0.9	2
too old/ ill to cultivate	0.9	2
tomatoes	0.9	2
sugar cane	0.9	2
potatoes	0.5	1
beans	0.5	1
pawpaw/ papaya	0.5	1
piri/ chili	0.5	1
sorghum	0.5	1
egg plant	0.5	1
cashew nuts	0.5	1
guavas	0.5	1

kankoma/ soy beans	0.5	1

Maize plays a big role in consumption since it is the main ingredient of the national dish *nsima*. In Figure 5-22 it can be seen how a woman is separating the corn from the cob.

Figure 5-22 A woman separating the corn from the cob to store it later in the big bags: the number of bags suggests that this is a very wealthy household

The local cotton production uses a lot of pesticides. The frequency of their application in intervals of 1-3 weeks can be seen in Figure 5-23.

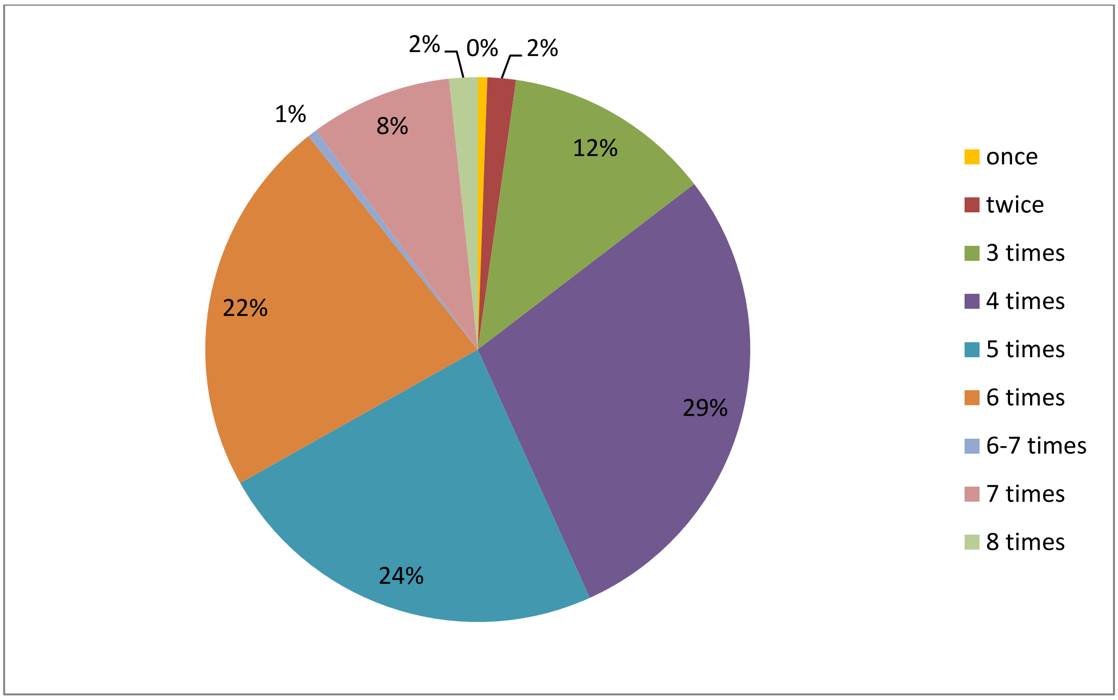


Figure 5-23 Application pattern of pesticides in cotton growing households

(n = 175, with intervals of 1 to 3 weeks between pesticide applications)

Cotton production is the responsibility of everybody in the household and even the children are involved as can be seen in Figure 5-24.

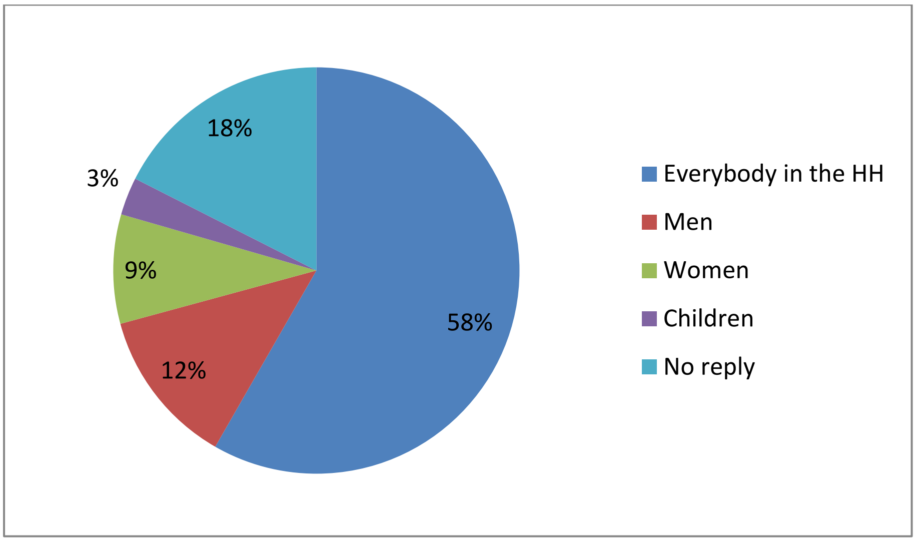


Figure 5-24 Cotton work distribution amongst household members

There are several cotton companies involved in the local production. The pattern of their contracting in the area can be seen in
Table 5-11.

Table 5-11 Frequency of contracts with cotton companies of the study area

Companies	No of households	Percentage of households
Single company		
Cargill	60	28.4
CC	7	3.3
Chipata Cotton	5	2.4
Clark cotton	2	1.0
Dunavant	47	22.3
Olam	45	21.3
Yustina	1	0.5
Subtotal: single companies	***167***	***96.5***
Multiple companies		
Cargill and Dunavant	1	0.5
Cargill and Olam	1	0.5
CC and Dunavant	1	0.5
CC and Olam	1	0.5
Clark cotton and Dunavant	1	0.5
Dunavant and Olam	1	0.5
Subtotal: multiple companies	***6***	***3.5***
No reply	38	18.0
Total	**211**	**100.0**

There have been a lot of disruptions due to cotton price management and changes. As a result, the farmers' earnings from cotton have been substantially reduced.

The market for the sale of crops and crop products has become worse than five years ago in the opinion of 57% of households, whereas 38% said it had improved.

5.5.12 Infrastructure of environment and wildlife observed

On question in relation to the ecosystem was if the vegetation had changed over the past five years. The answers are shown in Table 5-12. If the household stated that they had noticed a vegetation change, multiple answers were possible to describe in what way. Interestingly, about half of the households said they observed changes whereas the other half did not.

Table 5-12 Vegetation changes perceived by the households over the past 5 years

Vegetation change perceived	Percentage of respondents	Absolute no of answers	Categories (in %)
no	25.1	53	25.1
no reply	3.8	8	26.1
don't know	22.3	47	
yes- no further details	1.9	4	48.8
vegetation loss	15.1	46	
bigger trees	10.0	23	
overpopulation	8.8	20	
climate change	3.8	9	
more houses	3.4	14	
increased demand for charcoal/firewood	1.0	4	
vegetation does not grow taller	0.9	2	
more mangos	0.7	3	
more animals	0.5	2	
less snails, same amount of buffalo beans	0.5	1	
more vegetation	0.5	1	
poor soil fertility	0.5	1	
early burning culture	0.5	1	
increased farming activities	0.4	2	
good soil fertility	0.2	1	
less tsetse flies	0.2	1	
more human activities	0.1	1	
Total	**100.00**		**100.00**

Because of the proximity to the Luangwa national park, households would regularly observe wildlife in their surroundings. How often and what type of wildlife they see is presented in Table 5-13.

Table 5-13 List of wildlife species seen by the households

(Species with native name (in italics) could not be uniquely translated (see Text box 5-1) but a likely translation has been made.)

Wildlife species	**Percentage of households mentioning each species**
Monkey	28.8
Bushpig	24.0
Elephant	22.4
Impala	18.4
Bushbuck	18.4
Chikwiba (Bushbuck)	16.8
Kafundo (Grysbuck)	14.4
Hare	13.6
Kudu	11.2
Buffalo	9.6
Hyaena	7.2
Giraffe	6.4
Isha (duiker or impala)	5.6
Warthogs	3.2
Black lechwe	3.2
Zebra	3.2
Wildcat	3.2
Puku	2.4
Honey badger	1.6
Lion	1.6
Duiker	1.6
Porcupine	1.6
Wild dog	1.6
Genet	1.6
Eland	0.8
Hippo	0.8
Leopard	0.8
Crocodile	0.8
Vichuli (honey badger or frogs)	0.8
Simba (lion or civet)	0.8

Mbabala (Bushbuck)	0.8
Sanze (cane rat)	0.8
Antelope	0.8

Furthermore, when asked which species were most often in contact with wildlife, the answers indicated that it was mainly people and secondly dogs and goats (Figure 5-25).

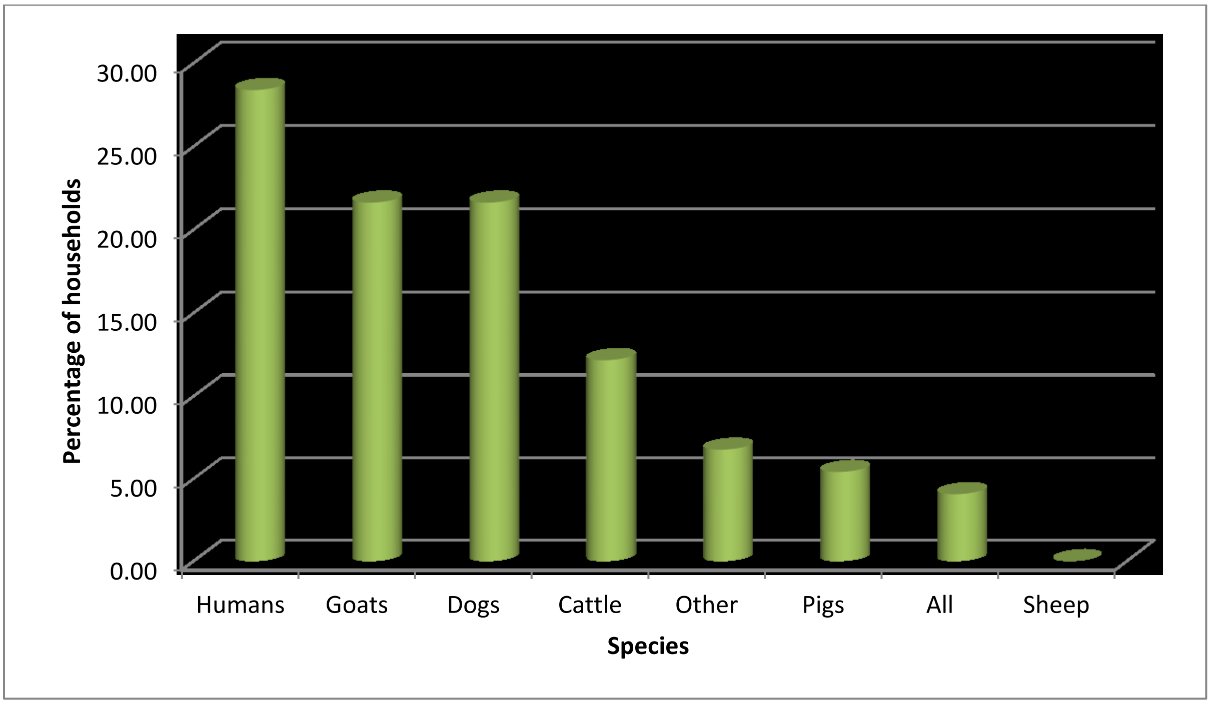


Figure 5-25 Species cited as most often encountering wildlife

The majority of wildlife contacts would be during the day (52%), followed by anytime (35%) and during the night (13%). There were possible several answers as where they would get in contact with them, so 18% households said at home, 22% said at the water source, 26% said while grazing, 46% said while in the bush and 19% mentioned other places such as the fields or the school yard.

5.6 Discussion

Since this study has been conducted in one region at one point in time, several calculations discussed in the general introduction cannot be done. This is also important to mention because the study area is highly prone to floods and droughts and this may influence the situation per year significantly (Freeman, 2008). Still, it is interesting to bring the different pieces of the puzzle together to be able to try to understand the big picture.

Demographically, there are hardly any changes since a survey was done in 1988. The average household size was around six at that time too and the percentage of marriage with 75% the same (Kalyocha G.C.K., 1988). The energy sources for lighting, cooking and heating reported in this study are as presented in the literature (ZAWA, 2011). The impact of the principal use of wood and charcoal on the ecosystem has been documented already (Dalal-Clayton and Child, 2003; Gumbo *et al.*, 2013). The influence that this change in ecosystem has on the disease situation has not been investigated.

The high use of bicycles may be an exception compared to other areas, but is easily explained by the presence of the bicycle company in Chipata. The use of a bike as transport mode shows some improvement compared to just walking and surely facilitates the exchange of goods and social networks within the area, which plays even a more important role since market infrastructure is scarce. The fact that radios are more often owned than mobile phones is especially important for any information campaigns (Seid *et al.*, 2016; Yepes *et al.*, 2016). The use of radios and mobile phones has also had a positive effect on household violence against women (Cardoso and Sorenson, 2017).

Historically the Luangwa Valley was never a livestock production area due to its high risk of trypanosomiasis (Carr, 1996; Dalal-Clayton and Child, 2003; Marks and

Fuller, 2008; Marks, 1976). However, the conclusion from this study is that livestock production is rising in the area. There is a high interest and demand from the people to improve their farming practices and increase their knowledge on livestock production. The handling of the animals during the survey has shown that progress on the understanding of animal behaviour and welfare is necessary. This was especially the case with dogs. A study from 2000 already mentioned the lack of veterinary extension services (Van den Bossche *et al.*, 2000). A possible contributing source for the lack of knowledge could be that even medical or veterinary practitioners in the area are not very knowledgeable in zoonotic diseases, as it was found in a study in Tanzania (John *et al.*, 2008). Personal observation confirms this point.

Chewa are traditionally more knowledgeable in agriculture and the exchange of ideas and advice between ethnicities could be beneficial for everybody. However, Chewa have an exceptional role in the study area since they appointed their own chief and they live in distinct places. This might be a sign for them to be treated as a minority and be less accepted by others.

The number of wildlife observed by the households and especially the high frequency of elephants seen, confirms the importance of human-wildlife-conflicts in this area. These conflicts exist for a long time and are increased by the intensification of agriculture (Kalyocha G.C.K., 1988). Dogs are needed for protection and to raise an alarm in case of incursion of wildlife.

A problem that was not in the main focus when planning this study was the role of women in society. There seem to be ethnic differences, since Kunda are matrilineal and seem to accept more often women in leading positions (ZAWA, 2011). This could also be observed looking at the number of households headed by women. There were more where Kunda were the predominant tribe than with the other ethnicities. Female headed households were generally perceived as much better managed than male headed households (personal communication several people in the area). This gender inequity could possibly contribute to the difference in life satisfaction between men and women. More female empowerment is needed for the development of the area.

It was remarkable to see how many people over the age of 60 are living alone heading their own household, whereas in other households they were taken care of by the rest of the family. Very few had children or grandchildren staying with them to support them in their daily work activities.

The migration pattern in the Luangwa Valley is mainly due to the loss of soil fertility or for social reasons. This seems to contradict the motivations Anderson *et al.* have given (Anderson *et al.*, 2015). This study was not able to confirm an influx of people to the mid-Luangwa Valley (Anderson *et al.*, 2015), but the area directly around the touristic town Mfuwe was not part of this study. Nonetheless, the majority of people stay in the area and do not leave the Eastern Province. The seasonal migration that some households have to do to cultivate their fields has a bad impact on their health and it should be investigated if this could not be avoided. Traditional rulers should be included into this discussion.

The education level is not very high in the study area, as expected from the literature (Project-Luangwa). The literacy rate of 39% fits well with the one reported in national census documents. But here also the gender difference is striking and girls should receive more support to attend school.

The risk factors for ill health are high in both in humans and animals. Animals seem to have diseases that have not been investigated so far, especially looking at their free range status, the high tick burden and the high number of abortions reported. Also the high number of humans with fits observed and reported cannot be explained given the relatively high presence of toilets. However, as Sagan and Kalyocha say the presence of infrastructure may be not useful without any education (Kalyocha G.C.K., 1988; Sagan, 1987). Toilet huts are free to be entered by animals such as pigs and it is not clear what people use to clean themselves and if they wash their hands afterwards. Sanitation is also generally not of high priority to the communities (Manase *et al.*, 2001). Their concerns are not related to health but about privacy, convenience, status, aesthetics and affordability (Manase *et al.*, 2001). The low amount of hygiene training for animal keeping and humans is striking and this would explain many of the disease occurrences reported. Just the cases of diarrhoea alone should be able to be reduced by increased adoption of more hygienic practices. This lack of knowledge also leads to the fact that people are not even aware they are engaging in any risky behaviour. On the other hand, the question what they would do with a little bit more money included the purchase of hygiene products. So, the question is if hygiene may be influenced by poverty as well, if soap is simply too expensive to buy.

The water quality in the area is bad. Nationally, 6.7% use water from unsafe sources (Manase *et al.*, 2001), but in the area it is more. Water quality, quantity and accessibility for human and animal consumption are strongly seasonally dependant. Some households even reported salty or coloured water, probably contaminated with manganese, but they do not have the option of not consuming it. There is very little maintenance of wells and boreholes in the area but it is not clear if this is due to a lack of knowledge or interest or defined responsibility.

Tsetse flies are seen less often and mainly observed during the rainy season, which corresponds with the findings in the other chapters of this thesis and the literature (Van den Bossche and De Deken, 2002). However, only a third of the people regularly sleep under insecticide treated bed nets and a fifth of the population have observed ticks on their bodies. These are indicators for the presence of vector-borne diseases and their related lack of protection and more investigation needs to be conducted. This is further reinforced by the fact, that about a quarter of human fever cases do not respond to malaria treatment and that 13% of all households always have a case of fever. Also the risk of rabies infection is extremely high since 10% of households reported dog bites followed by death. These are alarming figures and the true burden in the area needs to be examined. Thus there is evidence of the occurrence of several neglected diseases and this study highlights the likelihood of their occurrence with an attendant important negative impact on people's livelihoods. More importantly, the risk factors investigated are all related to diseases with a high

DALY burden (rabies, cysticercosis, trypanosomiasis, tick-borne infections, brucellosis).

In addition to that, more than half of the households report a decrease of food availability over the past five years. This is in agreement with the findings of Freeman (Freeman, 2008). It may be already an effect of the ecosystem change and a loss of soil fertility. The replies to the question that asked what people would do with a small amount of additional money, clearly demonstrated that food variety is missing and this also may explain the 11% of reported malnutrition in the households. The rate of malnutrition is even higher than the one reported earlier (Freeman, 2008). Meat is rarely eaten and the main protein source is groundnuts. Most of the households own poultry and their impact has not been directly accounted for in this study, but is reflected in people's replies about food. A more developed milk production in their livestock could possibly also alleviate some of malnourishment observed and reported.

The handling of aborted material was also very risk-prone since a significant number of households would do nothing, eat it (if this means human consumption is not clear) or give it to their dogs. These practices provide ideal conditions for the transmission and spread of possible infections. Although no positive serology was found for brucellosis in this study, in Nigeria feeding aborted materials to dogs has been found to be a risk factor for *Brucella* transmission (Cadmus *et al.*, 2011) and might play a role for the transmission of possible other diseases. The fact that nearly everybody lets their animals roam freely adds to this risk.

The results of the economic and health evaluations do not look optimistic but nevertheless, people reported a high level of life satisfaction. This paradox was already mentioned by Murray and Chen (Murray and Chen, 1992). It might be that their expectations are just so low that they are happy despite their situation. The changes in lifestyle are mainly thought by the people to come through government activities. Furthermore, the objective measures (looking at the assets) are low and thus do not correspond with the subjective measures (reported feelings). This contradicts the theory of Dolan *et al.* and Tinkler and Hicks (Dolan *et al.*, 2008; Tinkler and Hicks, 2011).

Looking at the range of individual household's farmed acreages and the sizes of their maize storage, huge differences among the households sampled become obvious. This may be a sign of economic difference even in an area that is perceived as uniformly poor from an outsider's viewpoint. The big differences in herd sizes, especially in cattle, fortify this theory.

The infrastructure of the health system as well as of the veterinary services is far away from the fulfilment of universal health coverage (WHO, 2017b). For animals more than for humans, people were willing to pay for private health services. A system for selling and distributing veterinary drugs and vaccines efficiently and with advice about the right dosage is desperately needed. It has to be noted that the tsetse control assistants in the area are not actually allowed to sell drugs, but without their illegal activity in providing these the situation would be worse. They do not have an animal health education, but the expectations of them from farmers are high. In addition, government workers lack transport and funds to visit the farmers or to undertake any interventions. The concept of public-private partnerships is not implemented in this area. There are no private veterinarians or animal health workers who could support the government structures.

The use and mention of mainly three drugs, two against trypanosomiasis and one antibiotic, shows the narrow focus and limited expertise and infrastructure in this area. This is however not a new fact; Van den Bossche *et al.* found out that preference is given to diminazene aceturate. The drug is given to any animal with clinical signs no matter if possible trypanosomiasis or not. The usage was highest during the dry season which seems to imply that most clinical cases occur then (Van den Bossche *et al.*, 2000). From some comments one could understand that animals and humans are taking the same drugs, which is not ideal. The risk of antimicrobial resistance (AMR) is extremely high and this problem should be tackled as soon as possible. Despite the inappropriate use of the drugs in the study mentioned above, the risk of resistance was considered low. It would be interesting to see how this has developed over time.

Keeping livestock means having an income, food and traction power. Since these are items clearly missing in the Luangwa Valley, it makes most sense to support an improved and more efficient, climate-friendly and ecological livestock production. Pigs were introduced by an NGO in order to have another smaller and affordable species available. Nonetheless, the high pig mortality implies that the management of this species is not well done. A good management would also imply quarantine measures for their pigs when an outbreak of African swine fever is known and they occur frequently in the area (Simulundu *et al.*, 2017). Dogs also have a high death rate, but that corresponds to a general life expectancy of only two to three years in African dogs (Kaare and Cleaveland, 1997; Kitala *et al.*, 2001). Therefore, dogs are bought regularly new and come as intake into the household. For cattle, there is evidence that animals are being brought in to the area, probably for draft. The calving rate of 60% is quite high compared to other areas, but Perry *et al.* found a similar rate in Katete (Perry *et al.*, 1984). In some cases the age of the animal was given by the owners, but often also determined by examining their teeth. Recollection bias is therefore unlikely. There are hardly any market outlets for livestock or related products and crops. This really limits the benefits that the households can generate from their animal husbandry activities (Randolph *et al.*, 2007). However, animal traction plays an important role and even female cattle are used for work.

The frequent usage of pesticides and the continuous clearing of new areas because of the loss of soil fertility is alarming. This might lead to a reduction in tsetse abundance which seems positive, but current negative and other long-term effects are ignored. The use of pesticides is hypothesised to have an effect on tsetse population. So if people stop growing cotton because of reduction of price, what would be the effect on flies, etc.?

Cotton production has increased since 1988 from 45% to 83% (Kalyocha G.C.K., 1988). It is very labour intensive and even children are involved in that work. The majority of households have a contract with a single cotton company which decreases competition and increases their dependence on the price scheme and the conditions imposed by that company.

The occurrence and impact of wildlife into this whole dynamic needs more investigation. The species mentioned were mainly seen during the day, which is when people are about and it is easiest to spot them. Also the species mentioned are mostly those big enough to be seen even from far away. Since wildlife is considered a reservoir for many diseases it would be interesting to see if their diseases status compares to the results found in this thesis.

In relation to the choice of land, traditional rulers have less influence nowadays. It would be interesting to know if the power to choose which land to settle on and farm is now more with the people or how the system had changed.

5.7 Conclusion

The assessment of the wellbeing of the people in the study area is based on multiple factors with complex dynamics.

Both humans and animals are regularly exposed to a range of diseases, through vectors, hygiene practices etc. and the capacities of managing the diseases in humans and animals are low. Economically, people are still on a very basic level. The education status is not very high and women especially are disadvantaged in several ways. Water quantity, quality and accessibility are not sufficient all year round. Food availability and variety is also a problem for many households. Livestock production is a very important means to improve the situation, but the provision of even the most basic veterinary services, drugs and training are urgently needed.

Corruption is a problem in the area in the sense that people are provided with benefits of receiving fertilizer for example when he is a friend of the person selling it. This is also the case with the private sale of hospital drugs.

Since the Zambian government influences the education system, the health system and crop prices, its impact on the lifestyle of the valley people is immense. This is reflected by their expectations to the government and seems not to have been addressed properly for a long time.

Chapter 6: General discussion

In general the work conducted as part of this investigation was well received in the study area. The people were cooperative and very grateful for any interest in their health and the problems they had to deal with every day. It has been emphasised that they should keep the expectations for any changes low, however, since the DDDAC project did not budget for any interventions or follow-up studies to take place.

6.1 Summary of work undertaken in this thesis

Between June and August 2013 a cross sectional survey was conducted in 210 households using a One Health approach. During this survey, animals were blood sampled for trypanosomiasis, tick-borne infections (of the type *Theileria*, *Babesia*, *Ehrlichia, Anaplasma* and *Rickettsia*) and brucellosis. Selected pigs were also sampled for African swine fever and cysticercosis. The species sampled were cattle, goats, sheep, pigs and dogs. At the same time, humans were blood sampled for malaria and human African trypanosomiasis. For all individuals, including people and animals, a questionnaire was completed regarding their health status, risk factors and general wellbeing (people only).

The results have been in many ways as expected such as the general poverty of the area, the inefficiency of health and veterinary services and the dependence of people's livelihoods on the environment and the climate. Nevertheless, some of the results have been surprising, especially compared to former reports and literature. The pattern of trypanosomiasis in the area has changed significantly; *T. vivax* is now the predominant species in cattle. Tick-borne infections are far more frequent and not only represented by East Coast fever. All brucellosis samples were negative, however, the available diagnostic tests are not ideal yet (de Glanville *et al.*, 2017). African swine fever seems only a threat in sporadic but recurring outbreaks. The general health of the animals is not ideal and other conditions need to be taken care of. In humans, the situation is similar. The malaria prevalence was quite high despite all control efforts and it being a disease that the Zambian government focuses on. Human African trypanosomiasis could not be unambiguously detected in the survey. Several samples showed up positive for tick-borne infections and these have not been reported from the study area before.

Questionnaires showed that women are in an inferior position in the study area. They are less educated, have distinct health problems, are not able to take decisions such as on contraception on their own. The availability of water and nutrition is sub-standard and has a debilitating effect on the livelihoods of people and animals. The strong climate variations in terms of drought and flood and a general decline of the environmental resilience have a significant impact on the everyday life of the inhabitants of the Luangwa Valley. The constant threat through wildlife adds to the challenge of survival. However, questioned on their life satisfaction, most people were positive.

6.2 Interpreting the results with a One Health perspective

Drawing the threads together from the different topics looked at in this study, it has to be said that further analysis is intended for publications. Also it must be noted that no causal relationships can be made, because this would need longitudinal surveys that can observe and assess the social, economic and ecological changes and health problems in a timely and spatial manner. However, earlier studies in the literature have provided evidence for selected interdependencies and relationships that will be used to interpret the results of this study. Moreover, the developments in the study area can be seen as a cycle or very complex network, since changes happening in one characteristic have an influence (inhibiting or supporting) on another characteristic. So the described environmental changes have an influence on health, but then health may have an influence on the environment as well for example through prevention measures or an increase in migration into the area because of reduced health risks. It is therefore not a simple linear relationship that can be targeted with only one specific intervention (for example focussing on the health sector alone). Experience has shown that outbreaks of cholera, a common sapronosis, are not prevented on a long-term basis just focussing on the health interventions, but activities are needed to make the environmental conditions less convenient for the pathogen and re-establish the natural balance of the ecosystem (Lara *et al.*, 2011).

6.2.1 Ecological changes and health

To start with, environmental changes have clearly taken place over the last few years. Land use changes have been reported from the whole area. These are represented by people who change their plot of land. This was mainly due to loss of soil fertility and social reasons. The plot they leave is eroded and usually without trees or bushes, because trees and bushes have been cut for cultivation purposes. This increases general deforestation and soil erosion which is exacerbated through droughts and floods. These environmental changes also impact on the tsetse flies as they are exigent in relation to their habitat as they need a distinct temperature, humidity and soil quality to reproduce. They prefer shady places under bushes and shrubs and the reduction of tsetse flies within the study area was noted by the colleagues from the DDDAC project (personal communication Dr. N. Machila). In addition to that, those that were interviewed suggested that tsetse observations are made 50% on animals and 50% on humans, this gives a hint to a changing feeding pattern of the flies. Usually, they would prefer to feed on animals, but because their habitats are more fragmented than before, they use the alternative human host more frequently. This might result in an increase in cases of sleeping sickness in the human population. At the same time, these ecological changes can lead to water stagnation and create puddles in these areas that become an ideal breeding ground for mosquitoes for example. Through the coverage of these areas with grass, ticks find an ideal habitat as well.

The fact that *T. vivax* is now the predominant trypanosome species in the area compared with *T. congolense* in former times, could also be explained by the tsetse population dynamics because *T. vivax* is a species that can be transmitted by other biting flies as well. That means the infection is not completely dependent on tsetse flies being present in the area. Looking at tick-borne infections, the prevalence is high. Also the number of co-infections in animals is high, but the impact this may have is not clear (Jongejan *et al.*, 1986; Musisi *et al.*, 1984; Simuunza *et al.*, 2011).

This study reports the first detection of zoonotic tick-borne infections including *B. caballi* and *A. phagocytophilum* in humans and in animals in Mambwe District. It is unclear if these infections have been present in humans in the study area for some time or have appeared more recently. The signs of infection are not well known and may be missed by health personnel especially if the person tests positive for malaria and has a co-infection. An increase in tick human interactions could be due to a loss in biodiversity (Bernstein, 2014; Hudson *et al.*, 2006; Ridder, 2008). Biodiversity is a key indicator for a healthy ecosystem (Keesing *et al.*, 2010; Keesing *et al.*, 2006). Studies on mosquitoes and especially on ticks in the Luangwa Valley mainly date back to the 1970s and 1980s. A new entomological investigation should be considered looking at the evidence so far (Ferguson *et al.*, 2010; Lobo *et al.*, 2015; Tanga and Ngundu, 2010).

6.2.2 Wildlife and its role for generating an income

Wildlife still seems to be abundant, considering the reports on observed species and problems with human-wildlife conflicts. On the other hand, one could argue that there must be a reason that wildlife is coming closer to the dwellings of people. Or is it that people are coming closer to the habitats of the animals? The increase in tourism could definitely make a change to certain behaviours of wildlife, but also the increased hunting and poaching activities reported (ZAWA, 2011). The last wildlife census conducted reported more carcasses and fewer numbers of animals of certain species (ZAWA, 2011).

Wildlife are mainly seen as an income opportunity in the valley, or as a threat to the income since livestock casualties are not compensated by the government. This has been also expressed by Carr (Carr, 1996):

> “What most western style people do not understand about wildlife conservation as we see it, is that the very idea is

> completely foreign to African culture and we are trying to impose on them something antagonistic to their beliefs."

The idea of sharing wildlife revenues through community resource boards has been innovative and promising, but unfortunately did not work out in the valley. The use and abuse of power and responsibility has led to corruption and thus a loss of trust from the general population (Pope, 2005). The benefits have not arrived where they were supposed to arrive and the population had to find a solution in generating money on their own. The most obvious way to do that would be by increasing the efficiency of their agricultural activities.

6.2.3 Crop production and its influence on health and poverty

Looking closer at the agricultural properties of the area, it becomes clear that a more sustainable and environmental-friendly way of cultivation and livestock keeping has to be applied. A mere increase in acreage and number of animals would not fulfil this nor the expectations that the farmers have. The crop production needs training, implementation and evaluation of adapted methodologies. For example the cotton production uses a lot of agricultural inputs which is neither safe for the ecosystem nor for the people using it (EJF, 2017). In Asia, the cultivation of organic cotton has reduced the chemicals needed and often seeds from more resilient plant species may not yield the same harvest results, but are better adapted to the growing conditions (Bachmann, 2012). Cotton is also a very demanding crop in terms of nutrients and water usage and ideally, crop rotation should be applied. To increase the profitableness of cotton, it could be an option to create a cooperative of many farmers, since these networks are increasing in the area, where some of them grow the cotton and others help processing it. The value of cotton increases once it is processed and this has been done in Zambia in former times. This way, crop rotation could be possible to the general benefit of everybody. Furthermore, there are several souvenir shops around Mfuwe who could easily trade the products made by organic farming.

The drop in cotton prices beginning of the 2010 has had a significant impact on the scale of production in the Eastern Province. The question is now, if the reaction of the farmers of growing less cotton than before has an impact on tsetse fliy abundance and thus on trypanosomiasis prevalence.

Another example is maize production. Like cotton, maize is a very demanding crop with not many nutritional properties. Maize also benefits from crop rotation and the local maize is more resilient than the hybrid one. However, maize is a staple food in Zambia and high amounts are needed to satisfy the needs. The advantage is that the storage does not need any sophisticated methods. Other plants and vegetables could be introduced to the farmers to increase food variety and make them more resilient towards climatic conditions.

6.2.4 Livestock production and its role for generating an income

There is already a high interest in increasing the efficiency of livestock farming. It is important to say that the number of domestic animals kept is critical for this area and that attention has to be paid to keep a balance on livestock farming in a game management area. In a way, tsetse flies were a good tool for wildlife conservation because they kept the amount of livestock production down. The sharing of diseases between wild and domestic animals, the competition for water, feed and space and the natural enmity between certain species does not only have an impact on the animals itself but on the whole complexity of the ecosystem and thus the wellbeing on the people who are part of it. An increase in the number of domestic animals will need more resources, increase more conflicts with wildlife and may finally expulse wildlife from the area. This could lead to only limited space left for the wild animals and thus a decrease in population sizes in those species.

The development of local milk production in cattle, sheep or goats could fight issues such as malnutrition and hunger, but also increase the use of one animal. The concept of organic farming is very efficient in that is uses the outputs from one production line to feed another one. This concept has been poorly applied in the area due to a lack of knowledge. Chewa seem to be more successful in livestock farming looking at the size of their cattle herds and farms. They should be given the opportunity to share their practices and experiences and also learn better adapted methodologies. Their cattle are already used for animal traction purposes which increases their value for agriculture considerably. Also the application of regular vaccination campaigns (especially for Newcastle disease and rabies) would improve the wellbeing of animals and their owners substantially.

6.2.5 Animal health and human health systems

A basis for livestock production is the adherence to food quality, food safety and animal welfare requirements. These characteristics are usually met along the value chain through the team work of a farmer, his animals and a veterinarian. This interaction is not happening in the study area due to non-availability of good quality veterinary services. The significant impact this has on the livelihoods of the people in the area is seen by the high rate of malnutrition, the possibly high prevalence of neurocysticercosis and the bad management of livestock and their diseases.

This brings the discussion back to the health status observed in animals and humans. A lack of communication and cooperation between the health sector, the veterinary sector and the environmental sector is visible. Education on One Health could have a system-improving effect and it would save money (Mbugi *et al.*, 2012; Zinsstag *et al.*, 2007b). For this as well, traditional healers need to become part of the system, because they are important for the general population in terms of health and problem solving.

The importance of trypanosomiasis seems to be reduced based on the lower prevalence compared to previous studies. However, the study area is known for few sporadic cases of sleeping sickness with sometimes a chronic clinical picture and low parasitemia. Therefore the health system needs to be vigilant to detect new cases. However, the information retrieved from the literature suggests that the diagnostic capacity of health services is not only restricted by their equipment, but also by the amount of staff and their applied knowledge on diseases (Mwanakasale *et al.*, 2013).

Given the large number of infections that cause fever it would be best to improve the protocol for patients with fever. The proportion of households having always someone with fever is alarmingly high and the diagnostic approach from the clinical perspective needs to have a substantial critical mind. So far, treatment options used are very limited and no further investigations are done or in sleeping sickness cases only after a substantial amount of time. This loss of time may even expand in a case of sleeping sickness, because the patient is referred to one or several other clinics until s/he reaches a hospital that has permission to treat sleeping sickness cases. Then it would take an additional few days until the drug has been ordered from WHO and reached the hospital and the treatment can start. By that time, the patient might be in late stage or have died. Therefore the argument should be counted in to re-evaluate the protocol and maybe let the drug come to the patient and not the other way round (Mwanakasale *et al.*, 2013).

The lack of an investigative mind and resources can be also confirmed by the new identification of tick-borne infections in humans and possibly a much higher prevalence of neurocysticercosis in the area. The health system did not register that yet nor conduct any interventions. The high malaria burden questions the success of the national malaria programme implemented. The fact that malaria prevalence is calculated or assessed only for children under five years (Crowell *et al.*, 2013), creates a bias as adults might serve as reservoir and contribute to the continuous occurrence of cases. The RDT used in Zambia diagnoses only *Pl. falciparum* and therefore will not detect any other parasites which, in this study, represented around a third of all positive cases.

From the health system perspective, it is a challenge to improve the availability, quality and accessibility of services with a narrow national budget such as the GDP for Zambia. Provided the political will is there to improve, the transformation in real activities is difficult. An increase in public-private partnerships could bridge these problems until the economic development can cover the costs of the system alone (Lim, 2004).

6.2.6 Ecology and poverty

Reviewing other sources of income, the trade with fire wood and charcoal has to be mentioned. Relying on them as the only sources of energy has disastrous effects on the ecosystem and leads to deforestation, which brings us back to the start of this discussion (Gumbo *et al.*, 2013). The search for a reliable source of income causes the main disruptions in the area and the idea of a basic income should be considered (Basic Income Grant Coalition, 2009). On the other hand, the expectations towards the government are already quite high. In the past, it was mostly the government, directly or through development projects, that took care of any issues in the area. Times have changed now and with this partly the role of the government too.

The market opportunities within the area, even with the tourism sector should be assessed as a possibility, but nepotism needs to be avoided by any means. Meat that has passed food safety controls, vegetables, fruits and local plants such as baobab or masau could become a delicacy for the tourists in the lodges and expensive import of food could be avoided.

6.2.7 Social factors with impact on wellbeing

Female empowerment could also solve certain issues in household management, livestock keeping, hygiene, nutrition and health. Teenage pregnancy is a problem and may be caused by abuse or rape (Birbeck *et al.*, 2008; Harrison *et al.*, 2017). Efforts to make the society more equal should be fostered by the government since then less people will become ill (Arcaya *et al.*, 2015; Wilkinson and Pickett, 2006).

However difficult the situation might seem in the Luangwa Valley, the respondents of the questionnaire still scaled high on their general happiness. This could be explained by the aspiration level theory, that the gap between what people wish for and what they have is not big (D'Acci, 2011). The perception of how bad the situation is might be also different (Carr *et al.*, 2001; Murray and Chen, 1992).

6.3 Recommendations and future work

From a research perspective it would be interesting to investigate further the following topics:

- Epidemiological survey for tick-borne infections in humans, animals and ticks
- Epidemiological study on cysticercosis in pigs and humans, including an evaluation of the sanitary situation
- Survey on wildlife densities and number and convenience samples for disease detection
- Environmental investigation on water quality and quantity and how to improve the situation.
- Repeat a similar study during the rainy season when prevalence of vector-borne diseases is supposedly highest and pesticides are used; focus also on vectors
- Clarification on the occurrence and immunological role of co-infections in humans and animals in the area

In addition to that, trainings to improve the knowledge in the area could be provided:

- Conduct regular training programmes for health and veterinary staff on disease, animal production management and correct dosage and application of animal drugs; support the acquisition of licences to sell drugs
- Rabies training: dog handling and keeping, vaccination, involve people in community as observer, sensitizer and assistants

Finally service provision should be improved in all sectors:

- Plan regular visits/meetings/drive through by veterinary/health staff to meet people, supply drugs, give advice and training

Appendices

Appendix I Human health questionnaire

Human health questionnaire

A) QUESTIONNAIRE AND RESPONDENT IDENTIFICATION

1. Date
2. Name of interviewer
3. Name of interviewee
4. Sample ID
    a. Census area (N/S)
    b. HH number (001)
    c. Sample number per HH (001)
    d. Species (H)
    e. Age of the interviewee in years
    f. Sex of the interviewee (F/M)
5. Are you able to read and write?
    a. Yes
    b. No
    c. A little bit
6. Your Position in household :
    a. Head of household
    b. Wife of head of household
    c. Child of household head
    d. Parents or parents-in-law of head of household
    e. Other
7. We are especially interested if women have any particular health problems. We hope you don't mind us asking about your experiences having children.

B) FEMALE REPRODUCTIVE HISTORY

8. Female reproductive history
    a. If female, how many times have you given birth?
        - None
        - Number?
    b. If female, age first time giving birth
        - number
    c. if female, How many living babies did you give birth to?
        - Number
    d. If female, How many of your births were attended by medically trained staff (midwives, nurses or doctors)?
        - number
    e. if female, Have you had any miscarriages/abortions?
        - None
        - Number
    f. If female, are you pregnant or breast-feeding a baby?
        - Pregnant
        - Feeding baby

- ❖ both
- ❖ none

g. Do you use any type of birth control?
- ❖ Yes
- ❖ No

h. If yes, which one?
- ❖ Text

C) PERSONAL HEALTH AND HABITS

9. Do you feel sick, not well or show any symptoms at the moment?
   a. No
   b. Yes
   c. If yes
      - ❖ text
10. How often have you not been able to work or go to school during the past 12 months because of an illness?
    a. More than 2 weeks
    b. Less than two weeks
    c. Never
11. Have you had any medical treatments in the last 12 months?
    a. Yes
    b. No
    c. If yes, did you go to the health centre for it?
       - ❖ Yes
       - ❖ No, where did you get it from
    d. If yes, was this a regular medication?
       - ❖ Yes
       - ❖ No
       - ❖ If yes, what for
    e. If yes, was it medication for an acute disease?
       - ❖ Yes, what for
       - ❖ no
12. When did you receive the last vaccine (month and year, if possible)?
    a. Text
13. What was it for?
    a. text
14. Body condition of the person
    a. Overweight
    b. Normal
    c. slim
15. How often do you drink alcohol (Kachazu, beer etc)?
    a. Every day
    b. Every week
    c. Every month
    d. Never
16. How often do you smoke tobacco?
    a. Every day

b. Every week
c. Every month
d. Never

17. How often do you smoke marihuana?
    e. Every day
    f. Every week
    g. Every month
    h. Never
18. Have you ever found ticks attached to your body?
    i. Yes
    j. no
19. Do you use an insecticide treated bed net regularly?
    k. Yes
    l. No
    m. Sometimes

D) HOUSEHOLD HEALTH

20. How satisfied are you with your life on a scale from 1 to 10?
    n. Scale
21. Any remarks by the interviewer
    o. text

Appendix II Animal health questionnaire

Animal health questionnaire

1) Date
2) Interviewer
3) Sample ID
    a. Census area (N/S)
    b. HH number (001)
    c. Sample number per HH (001)
    d. Species (D/Fe)
    e. Age of the animal in months
    f. Sex of the animal (F/M)
    g. Castrated/neutered (c)
    h. If female, reproductive information of the animal
        - ❖ Pregnant
        - ❖ with suckling
    i. If female, number of living young ones born during lifetime
    j. If female, age first time giving birth
    k. If female, number of abortions if any
4) Body condition score of the animal
    a. F+/F/F-/M+/M/M-/L+/L/L-
5) Any symptoms to be seen/ reported?
    a. text
6) When was last treatment or vaccine received?
    a. Text
7) What was it for?
    a. Text
8) Who did it?
    a. Text
9) What did it cost?
    a. Number
    b. Don't remember
10) Are there ticks to be seen on this animal?
    a. Many
    b. Few
    c. None visible
11) Where are the ticks mainly found on the body?
    a. text
12) Colour of coat
    a. text
13) Does the animal feed on wildlife?
    a. Yes/no
14) Does the animal come into contact with wildlife?
    a. During day/at night/anytime/never
15) Any remarks by the interviewer
    a. text

Appendix III Poverty and health questionnaire

Poverty and health questionnaire

Initially, information about the project will be provided, the diseases in focus, species sampled and why it is done. It will be made clear, that there are no direct advantages for the farmers out of this project, but that their cooperation helps decision makers to plan ahead and improve their well-being in the long run.

After the introduction, each person/the household head has to sign an ethical consent form where he/she agrees to give samples of animals/humans and to answer the questionnaires. It should be made clear that this is voluntary.

1. Introduction

- 1.1. Date and time
  - a. Date and time
- 1.2. Interviewer
  - a. Text
- 1.3. Translator's name
  - a. Name
  - b. none
- 1.4. name of household head
  - a. text
- 1.5. Sex of HHH
  - a. F
  - b. M
- 1.6. Age of HHH
  - a. number
- 1.7. Name of respondent
  - a. text

2. Household information

- 2.8. How many adult men live in the household?
  - a. number
- 2.9. How many of them are married?
  - a. number
- 2.10. How many children live in the household?
  - a. number
- 2.11. How many adult women live in the household?
  - a. number
- 2.12. How many of them are married?
  - a. Number
- 2.13. Are all people living permanently in this household present today?
  - a. Yes
  - b. No
  - c. If no, who is not
    - ❖ Text

2.14. What is the predominant tribe in the HH?
   a. Text

3. **Education**

3.15. How many children are going to school?
   a. Number
3.16. Tick highest education level reached?
   a. None
   b. Primary
   c. Lower Secondary
   d. Upper Secondary
   e. Basic school
   f. College
   g. University and beyond
   h. other

4. **Health services**

4.17. How do you get to the nearest health centre?
   a. Walk
   b. Bike
   c. Car
   d. other
4.18. How long do you have to travel, using above mentioned transport, to the next hospital or health care centre or doctor?
   a. No of minutes
4.19. How much does it cost?
   a. For free
   b. other
4.20. Are you satisfied with the health services provided in your area?
   a. Yes, with all services
   b. Only with Specific ones, e.g. ante-natal care etc
   c. no
4.21. Would you be willing to pay for a better accessible health service?
   a. Yes, even for general services
   b. Yes, only for Specific services
   c. No
   d. Other
4.22. Did you ever receive health/hygiene training for humans?
   a. Yes
   b. No
4.23. Is there any member of the HH who died since August 2012?
   a. Yes
   b. No
   c. If yes, what was the sex?
      - F
      - M

- ❖ If F, did death occur while pregnant, during childbirth or 6 weeks after?

d. If yes, what was the age?
- ❖ Number

4.24. Was there ever someone in this HH bitten by a dog and died shortly afterwards?
a. Yes, with anti-rabies treatment
b. Yes, without treatment
c. Never

4.25. Was there ever someone in this HH who did not respond to malaria treatment?
a. Yes
b. No
c. If yes, what happened
- ❖ Text

4.26. How often do you experience cases of fever in this HH during the past 12 months?
a. Always someone
b. Sometimes
c. Rarely
d. Never
e. If yes, who is mainly affected
- ❖ Children
- ❖ Women
- ❖ Men
- ❖ all

4.27. How do you treat these fevers?
a. Go to health centre
b. Traditional healer
c. church
d. Self-medication
e. Nothing
f. other

4.28. How often have you experienced cases of diarrhoea in this HH during the past 12 months?
a. Always someone
b. Sometimes
c. Rarely
d. Never
e. If yes, who is mainly affected
- ❖ Children
- ❖ Women
- ❖ Men
- ❖ All

4.29. How do you treat these cases?
a. Go to health centre
b. Traditional healer
c. Self-medication

d. church
e. Nothing
f. Other

4.30. Do you know of any diseases that are shared between humans and animals?
a. Text
b. None
c. Not sure

5. Water and sanitation

5.31. What type of water access is available?
a. River
b. Well
c. Borehole
d. other

5.32. How long does it take to go there?
a. min

5.33. Is the water quality good for you?
a. Yes, always
b. Only in rainy season
c. Only in cold season
d. Only in hot season
e. No

5.34. Are there times when water is scarce?
a. Yes, always
b. In cold season
c. In hot season
d. No

5.35. Do you have a toilet that you regularly use?
a. Yes
b. No

5.36. Do you know people having fits or acting bewitched?
a. Many
b. Few
c. None
d. If yes, are there more than 10 years ago
   - Yes
   - No
   - Don't know

5.37. Are you keeping livestock?
a. Yes→6&7
b. No→8

6. If yes, Veterinary services

6.38. Are there veterinary services available to you in close proximity?
a. Yes

b. No
c. Regular visits
d. Irregular visits

6.39. What is the distance to the next veterinary facility?
a. km

6.40. Are there veterinary drugs available to you?
a. Yes
b. No
c. Sometimes or some drugs

6.41. Where do you get them?
a. text

6.42. What treatments for acute diseases have you done for your animals?
a. None
b. other

6.43. What prevention measures and vaccines are done regularly for your animals?
a. None
b. Deworming
c. other

6.44. What improvements would you like to see to current animal health care provision?
a. text

6.45. Would you be willing to pay for a better accessible (private) veterinary service?
a. Yes
b. No
c. Special services
d. other

6.46. Did you ever receive health/hygiene training for animal and/or animal production methods?
a. Yes
b. No
c. Long time ago

7. <u>If yes, Animal production</u>

7.47. Are all of these animals present today?
a. Yes
b. No
c. If no, which ones not
  - text

7.48. What is the purpose to keep animals?
a. Traction power
b. Value
c. Food
d. status
e. other

7.49. How many cattle do you own?

- ❖ number

7.50. How many goats do you own?
- ❖ number

7.51. How many sheep do you own?
- ❖ number

7.52. How many pigs do you own?
- ❖ number

7.53. How many dogs do you own?
- ❖ number

7.54. How many animals died within the past 12 months?
a. Cattle
b. Goats
c. Sheep
d. Pigs
e. Dogs
f. None
g. other

7.55. How many abortions, stillbirths or weak-born offspring have you observed in your animals during the past 12 months?
a. Cattle
- ❖ number

b. Goats
- ❖ number

c. Sheep
- ❖ number

d. Pigs
- ❖ number

e. Dogs
- ❖ number

f. None
g. Other

7.56. What do you do with the aborts and associated materials?
a. text

7.57. Did you give any animals as a gift or sale or in custodial care in the last 12 months?
a. Gift
b. Sale
c. Custodial care
d. No
e. If yes
- ❖ Number and species and type

7.58. Did you receive any animals as a gift or purchase or in custodial care in the last 12 months?
a. Gift
b. Purchase
c. Custodial care
d. No
e. If yes

- ❖ Number and species and type

7.59. How many animals were born within the past 12 months
  a. Cattle
    - ❖ number
  b. Goats
    - ❖ number
  c. Sheep
    - ❖ number
  d. Pigs
    - ❖ number
  e. Dogs
    - ❖ number
  f. None
  g. other

7.60. Do you own animals for traction?
  a. Yes
  b. no
  c. If yes, how many days were your animals used to plough the fields or other draft work by you or others?
    - ❖ number
  d. If yes, were there any times during the last 6 months when you could not use your draft cattle because they were sick?
    - ❖ Yes
    - ❖ No
    - ❖ If yes, how long?
    - ❖ Text

7.61. Who has the main ownership of the stock?
  a. HHH
  b. Other members of HH
  c. Other people not living in the HH
  d. other

7.62. Does tick infestation present a serious problem to your animals?
  a. Yes
  b. No
  c. Some species
    - ❖ which
  d. if yes, When are your animals most affected by tick infestation?
    - ❖ Dry season
    - ❖ Wet season
    - ❖ Cold season
    - ❖ Always
    - ❖ other

7.63. Do you use any measures to control ticks in your animals? GRID QUESTION IN SOFTWARE
  a. Yes
  b. No
  c. If yes, what
    - ❖ Pour-on

- ❖ Spraying
- ❖ Hand-picking the ticks of the animal
- ❖ Nothing
- ❖ Other

d. If yes, which species
- ❖ Cattle
- ❖ Goats
- ❖ Sheep
- ❖ Pigs
- ❖ Dogs
- ❖ Other
- ❖ All

e. If yes, how often
- ❖ Regularly
- ❖ Rarely

7.64. Are there any ticks found in the direct environment of the animals (kraals etc)?
a. Yes
b. No
c. sometimes

7.65. Do you graze your animals with other households' herds?
a. Yes
b. No
c. Sometimes
d. Free-ranging

7.66. Do you allow male animals from other people mate with your female animals?
a. Yes
b. No
c. Free-ranging

7.67. Do you allow your male animals to mate with female animals from other people?
a. Yes
b. No
c. Free-ranging

7.68. Did you have enough to feed your animals during the past 5 years?
a. Yes
b. No
c. Some years

8. <u>Fauna/wildlife & contacts with them</u>

8.69. Do you regularly see wildlife in your area?
a. Yes
b. No
c. If yes, which species
- ❖ text

8.70. Do you or your animals have contact with wildlife?
- a. Often/regularly
- b. More in dry season
- c. More in rainy season
- d. Rarely
- e. never
- f. If yes
  - ❖ During the night
  - ❖ During the day
- g. If yes, which species?
  - ❖ Cattle
  - ❖ Goats
  - ❖ Sheep
  - ❖ Pigs
  - ❖ Dogs
  - ❖ humans
  - ❖ All
  - ❖ other
- h. If yes, where?
  - ❖ At home
  - ❖ At water source
  - ❖ While grazing
  - ❖ While hunting/in the bush
  - ❖ Other

8.71. Do you see regularly tsetse flies on the animals or people?
- a. Animals
- b. People
- c. Both
- d. Rarely
- e. seasonally
- f. none

8.72. How far away is the area with tsetse flies from your home?
- a. Min of walking

9. <u>Crop/vegetable production</u>

9.73. Would you say that the vegetation in your area has changed over the past 5 years?
- a. Yes
- b. No
- c. Don't know

9.74. Which crops/vegetables are farmed?
- a. text

9.75. Do you grow cotton?
  - ❖ Yes
  - ❖ No
  - ❖ Not anymore
  - ❖ Will do in future

a. If yes, how many minutes from your home is cotton grown?
- min

b. Who is growing the cotton?
- Men
- Women
- Children
- All

c. If yes, which company are you with

d. text

9.76. How many days did you use someone's cattle for ploughing or other draft work?

a. Number

b. Did not use

9.77. How often do you use pesticides on cotton?

a. X times per X

b. X I

9.78. To whom do you sell your animal products?

a. Not selling

b. other

9.79. Do you produce and/or consume milk and milk products from your own animals?

a. Yes

b. No

c. If yes, do you treat these products before consumption?
- How
- No

10. <u>Migration</u>

10.80. Have you moved into this area during the past 10 years?

a. Yes

b. no

c. if yes, What were the reasons?
- More land
- More fertile land
- Want to keep livestock
- Join other people
- other

d. From where did you come?
- Text

e. What is better here than where you were?
- Text

f. What is worse here than where you were?
- text

10.81. Why are you living where you live?

a. Someone told me to live here

b. I chose the place on my own

c. I inherited the place
d. other

10.82. Do you move every year during the rainy season to the fields?
a. Yes
b. no
c. if yes, how far is it away and in which direction
- km
- north
- south
- east
- west

d. if yes, Do you feel that yearly migration affects the health of
- Animals
- Humans
- Both
- None

11. Economic status/poverty

11.83. Total acreage of farm?
a. Number

11.84. Do you have a maize storage?
a. Yes
b. No
c. If yes, how many 50kg bags fit into your maize storage?
- text

11.85. Which functioning household assets do you have in your household? GRID QUESTION IN SOFTWARE
a. mobile phone
b. bicycle
c. motor vehicle
d. motor cycle
e. solar panel
f. Television
g. Radio
h. Plough
i. Wheelbarrow
j. Scotch cart
k. sleigh
l. refrigerator/freezer
m. sofa

11.86. What is the main source of energy used for lighting, cooking and heating? GRID QUESTION IN SOFTWARE
a. Electricity
b. Gas
c. Wood
d. Candle
e. Paraffin

f. Charcoal
g. Solar
h. None
i. Other

12. Resilience

12.87. As compared to 5 years ago, would you say that :
Your household has a
a. more,
b. less or
c. equal
amount of food ?

12.88. Are there any malnutrition or deficiency problems in your household that you are aware of?
a. Yes
b. No
c. sometimes

12.89. What groups, organisations or clubs do you belong to?
a. Name
b. None

13. Value setting

13.90. What of the things you possess has the highest value for you?
a. Food
b. Water
c. Animal feed
d. Animals
e. House and land
f. other

13.91. What disease would you wish to control in your livestock if money was available?
a. text

13.92. What would you do if you had 50 KMW more per month?
a. text

13.93. As compared to 5 years ago, would you say that : Your household has
a. more,
b. less or
c. same
savings available to cope with illness or other problems ?

13.94. As compared to 5 years ago, would you say that :
There is a
a. increase,
b. decrease or
c. no change
in access to family/community support network ?

13.95. As compared to 5 years ago, would you say that :

The market for the sale of your livestock and related products is in general
a. better,
b. worse or
c. the same ?

13.96. As compared to 5 years ago, would you say that :
The market for the sale of your crops and related products is in general
a. better,
b. worse, or
c. the same ?

13.97. As compared to 5 years ago, would you say that :
In general do you think your lifestyle is
a. better,
b. worse or
c. the same ?

13.98. If worse or the same, what would you like to change to improve your lifestyle?
a. Text

13.99. What do you feel is the responsibility of the government in animal health care?
a. text

13.100. What do you feel is the responsibility of the private sector providers in animal health care?
a. text

13.101. What do you feel is the responsibility of the farmers/animal owners in animal health care?
a. Text

14. <u>Conclusion</u>

14.102. Do you have any questions or comments for us?
a. text

14.103. Outward appearance on a scale of 1-4
a. Condition of clothes
b. personal hygiene
c. dental health

14.104. Time interview was finished
a. Time

14.105. Any remarks by the interviewer
a. text

References

AANENSEN, D. M., HUNTLEY, D. M., FEIL, E. J. & SPRATT, B. G. (2009) EpiCollect: linking smartphones to web applications for epidemiology, ecology and community data collection. *PLoS One,* 4, e6968.

ABENGA, J., DAVID, K., EZEBUIRO, C. & LAWANI, F. (2004) Observations on the tolerance of young dogs (puppies) to infection with Trypanosoma congolense. *African Journal of clinical and experimental Microbiology,* 6, 28-33.

ABERNETHY, D. A., MENZIES, F. D., MCCULLOUGH, S. J., MCDOWELL, S. W., BURNS, K. E., WATT, R., GORDON, A. W., GREINER, M. & PFEIFFER, D. U. (2012) Field trial of six serological tests for bovine brucellosis. *Vet J,* 191, 364-70.

ACEVEDO-WHITEHOUSE, K. & DUFFUS, A. L. (2009) Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 364, 3429-3438.

ACHIA, T. N. (2015) Tobacco use and mass media utilization in sub-Saharan Africa. *PLoS One,* 10, e0117219.

AFONSO, S. M., VAZ, Y., NEVES, L., PONDJA, A., DIAS, G., WILLINGHAM, A. L., VILHENA, M., DUARTE, P. C., JOST, C. C. & NOORMAHOMED, E. V. (2011) Human and porcine Taenia solium infections in Mozambique: identifying research priorities. *Anim Health Res Rev,* 12, 123-9.

AGBOR, V. N., MBANGA, C. M. & NJIM, T. (2017) Adolescent deliveries in rural Cameroon: an 8-year trend, prevalence and adverse maternofoetal outcomes. *Reprod Health,* 14, 122.

AGUIRRE, A. A., BEASLEY, V. R., AUGSPURGER, T., BENSON, W. H., WHALEY, J. & BASU, N. (2016) One health-Transdisciplinary opportunities for SETAC leadership in integrating and improving the health of people, animals, and the environment. *Environmental toxicology and chemistry,* 35, 2383-2391.

AIT LBACHA, H., ALALI, S., ZOUAGUI, Z., EL MAMOUN, L., RHALEM, A., PETIT, E., HADDAD, N., GANDOIN, C., BOULOUIS, H. J. & MAILLARD, R. (2015) High Prevalence of Anaplasma spp. in Small Ruminants in Morocco. *Transboundary and emerging diseases*.

ALI, S., GUGLIEMINI, O., HARBER, S., HARRISON, A., HOULE, L., IVORY, J., KERSTEN, S., KHAN, R., KIM, J., LEBOA, C., NEZ-WHITFIELD, E., O'MARR, J., ROTHENBERG, E., SEGNITZ, R. M., SILA, S., VERWILLOW, A., VOGT, M., YANG, A. & MORDECAI, E. A. (2017) Environmental and Social Change Drive the Explosive Emergence of Zika Virus in the Americas. *PLoS Negl Trop Dis,* 11, e0005135.

ALLSOPP, M. T., HATTINGH, C. M., VOGEL, S. W. & ALLSOPP, B. A. (1999) Evaluation of 16S, map1 and pCS20 probes for detection of Cowdria and Ehrlichia species. *Epidemiol Infect,* 122, 323-8.

ALTIERI, M. A. (1999) The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems & Environment,* 74, 19-31.

ANDERSON, N. E., MUBANGA, J., FÈVRE, E. M., PICOZZI, K., EISLER, M. C., THOMAS, R. & WELBURN, S. C. (2011) Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis,* 5, e1211.

ANDERSON, N. E., MUBANGA, J., MACHILA, N., ATKINSON, P. M., DZINGIRAI, V. & WELBURN, S. C. (2015) Sleeping sickness and its relationship with development and biodiversity conservation in the Luangwa Valley, Zambia. *Parasites & vectors,* 8, 224.

ARCAYA, M. C., ARCAYA, A. L. & SUBRAMANIAN, S. (2015) Inequalities in health: definitions, concepts, and theories. *Global health action,* 8, 27106.

ASHLEY, S., HOLDEN, S. & BAZELEY, P. (1999) *Livestock in poverty-focused development*, Livestock in Development Crewkerne, UK

ASSANA, E., LIGHTOWLERS, M. W., ZOLI, A. P. & GEERTS, S. (2013) Taenia solium taeniosis/cysticercosis in Africa: risk factors, epidemiology and prospects for control using vaccination. *Vet Parasitol,* 195, 14-23.

ATLAS, R., RUBIN, C., MALOY, S., DASZAK, P., COLWELL, R. & HYDE, B. (2010) One Health-attaining optimal health for people, animals, and the environment. *Microbe,* 5, 383-389.

AUTY, H., ANDERSON, N. E., PICOZZI, K., LEMBO, T., MUBANGA, J., HOARE, R., FYUMAGWA, R. D., MABLE, B., HAMILL, L., CLEAVELAND, S. & WELBURN, S. C. (2012) Trypanosome diversity in wildlife species from the serengeti and Luangwa Valley ecosystems. *PLoS Negl Trop Dis,* 6, e1828.

BACHMANN, F. (2012) Potential and limitations of organic and fair trade cotton for improving livelihoods of smallholders: evidence from Central Asia. *Renewable Agriculture and Food Systems,* 27, 138-147.

BAKER, M. C., MATHIEU, E., FLEMING, F. M., DEMING, M., KING, J. D., GARBA, A., KOROMA, J. B., BOCKARIE, M., KABORE, A., SANKARA, D. P. & MOLYNEUX, D. H. (2010) Mapping, monitoring, and surveillance of neglected tropical diseases: towards a policy framework. *Lancet,* 375, 231-8.

BANAI, M., ADAMS, L., FREY, M., PUGH, R. & FICHT, T. (2002) The myth of Brucella L-forms and possible involvement of Brucella penicillin binding proteins (PBPs) in pathogenicity. *Veterinary microbiology,* 90, 263-279.

BARRETT, M. P. (2006) The rise and fall of sleeping sickness. *The Lancet,* 367, 1377-1378.

BARRETT, M. P., BURCHMORE, R. J., STICH, A., LAZZARI, J. O., FRASCH, A. C., CAZZULO, J. J. & KRISHNA, S. (2003a) The trypanosomiases. *The Lancet,* 362, 1469-1480.

BARRETT, M. P., BURCHMORE, R. J., STICH, A., LAZZARI, J. O., FRASCH, A. C., CAZZULO, J. J. & KRISHNA, S. (2003b) The trypanosomiases. *Lancet,* 362, 1469-80.

BASIC INCOME GRANT COALITION (2009) Making the difference! The BIG in Namibia-Basic Income Grant Pilot Project Assessment Report, <http://www.bignam.org/Publications/BIG_Assessment_report_08b.pdf>.

BATE, R., COTICELLI, P., TREN, R. & ATTARAN, A. (2008) Antimalarial drug quality in the most severely malarious parts of Africaâ€"a six country study. *PLoS One,* 3, e2132.

BECK, S., PULKKI-BRAENNSTROEM, A.-M. & SAN SEBASTIAN, M. (2015) Basic income-healthy outcome? Effects on health of an Indian basic income pilot project: a cluster randomised trial. *Journal of Development Effectiveness,* 7, 111-126.

BEKKER, C. P., DE VOS, S., TAOUFIK, A., SPARAGANO, O. A. & JONGEJAN, F. (2002) Simultaneous detection of Anaplasma and Ehrlichia species in ruminants and detection of Ehrlichia ruminantium in Amblyomma variegatum ticks by reverse line blot hybridization. *Vet Microbiol,* 89, 223-38.

BENGALY, Z., SIDIBE, I., GANABA, R., DESQUESNES, M., BOLY, H. & SAWADOGO, L. (2002) Comparative pathogenicity of three genetically distinct types of Trypanosoma congolense in cattle: clinical observations and haematological changes. *Veterinary parasitology,* 108, 1-19.

BENGIS, R., KOCK, R. & FISCHER, J. (2002) Infectious animal diseases: the wildlife/livestock interface. *Revue Scientifique et Technique-Office international des epizooties,* 21, 53-66.

BERKVENS, D. L., GEYSEN, D. M., CHAKA, G., MADDER, M. & BRANDT, J. R. (1998) A survey of the ixodid ticks parasitising cattle in the Eastern province of Zambia. *Med Vet Entomol,* 12, 234-40.

BERNSTEIN, A. S. (2014) Biological diversity and public health. *Annual review of public health,* 35, 153-167.

BHATT, S., WEISS, D. J., CAMERON, E., BISANZIO, D., MAPPIN, B., DALRYMPLE, U., BATTLE, K., MOYES, C. L., HENRY, A., ECKHOFF, P. A., WENGER, E. A., BRIET, O., PENNY, M. A., SMITH, T. A., BENNETT, A., YUKICH, J., EISELE, T. P., GRIFFIN, J. T., FERGUS, C. A., LYNCH, M., LINDGREN, F., COHEN, J. M., MURRAY, C. L. J., SMITH, D. L., HAY, S. I.,

CIBULSKIS, R. E. & GETHING, P. W. (2015) The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature,* 526, 207-211.

BIGGS, B., KING, L., BASU, S. & STUCKLER, D. (2010) Is wealthier always healthier? The impact of national income level, inequality, and poverty on public health in Latin America. *Soc Sci Med,* 71, 266-73.

BIRBECK, G., CHOMBA, E., ATADZHANOV, M., MBEWE, E. & HAWORTH, A. (2007) The social and economic impact of epilepsy in Zambia: a cross-sectional study. *Lancet Neurol,* 6, 39-44.

BIRBECK, G. L., CHOMBA, E., ATADZHANOV, M., MBEWE, E. & HAWORTH, A. (2008) Women's experiences living with epilepsy in Zambia. *Am J Trop Med Hyg,* 79, 168-72.

BLASCO, J. M., GARIN-BASTUJI, B., MARIN, C. M., GERBIER, G., FANLO, J., JIMENEZ DE BAGUES, M. P. & CAU, C. (1994) Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of Brucella melitensis infection in sheep and goats. *Vet Rec,* 134, 415-20.

BOYD, C., BLENCH, R., BOURN, D., DRAKE, L. & STEVENSON, P. (1999) *Reconciling interests among wildlife, livestock and peolple in eastern Africa: a sustainable livelihoods approach*, ODI

BRANDT, J. R., GEERTS, S., DE DEKEN, R., KUMAR, V., CEULEMANS, F., BRIJS, L. & FALLA, N. (1992) A monoclonal antibody-based ELISA for the detection of circulating excretory-secretory antigens in Taenia saginata cysticercosis. *Int J Parasitol,* 22, 471-7.

BRATHWAITE, R., ADDO, J., SMEETH, L. & LOCK, K. (2015) A systematic review of tobacco smoking prevalence and description of tobacco control strategies in Sub-Saharan African countries; 2007 to 2014. *PLoS One,* 10, e0132401.

BROCK, P. M., FORNACE, K. M., PARMITER, M., COX, J., DRAKELEY, C. J., FERGUSON, H. M. & KAO, R. R. (2016) Plasmodium knowlesi transmission: integrating quantitative approaches from epidemiology and ecology to understand malaria as a zoonosis. *Parasitology,* 143, 389-400.

BROOKS, W. C. (2013) Ehrlichia Infection in Dogs [online]. www.veterinarypartner.com. Available from: http://www.veterinarypartner.com/Content.plx?A=2103 [June 2013]

BRUCELLOSIS, J. F. W. E. C. O. (1986) *Joint FAO/WHO Expert Committee on Brucellosis: Sixth Report*, World Health Organization

BRUNNER, E. (1997) Stress and the biology of inequality. *BMJ: British medical journal,* 314, 1472.

BUKACHI, S., WANDIBBA, S. & NYAMONGO, I. (2009) The treatment pathways followed by cases of human African trypanosomiasis in western Kenya and eastern Uganda. *Annals of Tropical Medicine & Parasitology,* 103, 211-220.

BULTERYS, P. L., MHARAKURWA, S. & THUMA, P. E. (2009) Cattle, other domestic animal ownership, and distance between dwelling structures are associated with reduced risk of recurrent Plasmodium falciparum infection in southern Zambia. *Tropical Medicine & International Health,* 14, 522-528.

BUSH, J. B., ISAACSON, M., MOHAMED, A. S., POTGIETER, F. T. & DE WAAL, D. T. (1990) Human babesiosis--a preliminary report of 2 suspected cases in South Africa. *S Afr Med J,* 78, 699.

BUYST, H. (1977) The epidemiology of sleeping sickness in the historical Luangwa valley. *Ann Soc Belg Med Trop,* 57, 349-359.

CABELLO, C. & CABELLO, C. (2008) Zoonoses with wildlife reservoirs: a threat to public health and the economy. *Revista medica de Chile,* 136, 385-393.

CADMUS, S. I., ADESOKAN, H. K., AJALA, O. O., ODETOKUN, W. O., PERRETT, L. L. & STACK, J. A. (2011) Seroprevalence of Brucella abortus and B. canis in household dogs in southwestern Nigeria: a preliminary report. *J S Afr Vet Assoc,* 82, 56-7.

CANNING, D. (2006) Priority setting and the neglected tropical diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 100, 499-504.

CARDOSO, L. A. (2012) Environmental and economic impacts of livestock productivity increase in sub-Saharan Africa. *Tropical Animal Health and Production,* 44, 1879-1884.

CARDOSO, L. F. & SORENSON, S. B. (2017) Violence Against Women and Household Ownership of Radios, Computers, and Phones in 20 Countries. *Am J Public Health,* 107, 1175-1181.

CARR, A. J., GIBSON, B. & ROBINSON, P. G. (2001) Measuring quality of life: Is quality of life determined by expectations or experience? *BMJ: British medical journal,* 322, 1240.

CARR, N. (1996) *Kakuli: A Story about Wild Animals, Their Struggle to Survive and the People who Live Among Them*, CBC Pub.

CARTWRIGHT, C. P. (1999) Utility of multiple-stool-specimen ova and parasite examinations in a high-prevalence setting. *Journal of Clinical Microbiology,* 37, 2408-2411.

CAZORLA, C. C., SOCOLOVSCHI, C., JENSENIUS, M. & PAROLA, P. (2008) Tick-borne diseases: tick-borne spotted fever rickettsioses in Africa. *Infectious disease clinics of North America,* 22, 531-544.

CBD. (1993) Text of the Convention [online]. Available from: https://www.cbd.int/convention/articles/default.shtml?a=cbd-02

CDC. (2011) Neglected Tropical Diseases [online]. Available from: http://www.cdc.gov/globalhealth/ntd/diseases/index.html [6th July 2015]

CDC (2016) Malaria biology.

CENTRAL STATISTICAL OFFICE ZAMBIA (2003) 2000 Census of population & housing: Summary report

CENTRAL STATISTICAL OFFICE ZAMBIA (2011) 2010 Census of population & housing: Preliminary population figures

CENTRAL STATISTICAL OFFICE ZAMBIA (2012a) 2010 Census of population & housing: Descriptive tables 11.

CENTRAL STATISTICAL OFFICE ZAMBIA (2012b) 2010 Census of population & housing: Population summary report

CHANDA, E., COLEMAN, M., KLEINSCHMIDT, I., HEMINGWAY, J., HAMAINZA, B., MASANINGA, F., CHANDA-KAPATA, P., BABOO, K. S., DÜRRHEIM, D. N. & COLEMAN, M. (2012a) Impact assessment of malaria vector control using routine surveillance data in Zambia: implications for monitoring and evaluation. *Malaria journal,* 11, 437.

CHANDA, E., KAMULIWO, M., STEKETEE, R. W., MACDONALD, M. B., BABANIYI, O. & MUKONKA, V. M. (2012b) An overview of the malaria control programme in Zambia. *ISRN preventive medicine,* 2013.

CHANDA, E., MASANINGA, F., COLEMAN, M., SIKAALA, C., KATEBE, C., MACDONALD, M., BABOO, K. S., GOVERE, J. & MANGA, L. (2008) Integrated vector management: the Zambian experience. *Malaria journal,* 7, 164.

CHIDUWA, G., CHIMONYO, M., HALIMANI, T., CHISAMBARA, S. & DZAMA, K. (2008) Herd dynamics and contribution of indigenous pigs to the livelihoods of rural farmers in a semi-arid area of Zimbabwe. *Tropical Animal Health and Production,* 40, 125-136.

CHIFUNGULA, A. O. (2014) Report of the auditor general on the management of wildlife. Republic of Zambia.

CHIMA, R. I., GOODMAN, C. A. & MILLS, A. (2003) The economic impact of malaria in Africa: a critical review of the evidence. *Health policy,* 63, 17-36.

CHIMANA, H. M., MUMA, J. B., SAMUI, K. L., HANGOMBE, B. M., MUNYEME, M., MATOPE, G., PHIRI, A. M., GODFROID, J., SKJERVE, E. & TRYLAND, M. (2010) A comparative study of the seroprevalence of brucellosis in commercial and small-scale mixed dairy-beef cattle enterprises of Lusaka province and Chibombo district, Zambia. *Trop Anim Health Prod,* 42, 1541-5.

CHINYOKA, S., DHLIWAYO, S., MARABINI, L., DUTLOW, K., MATOPE, G. & PFUKENYI, D. M. (2014) Serological survey of Brucella canis in dogs in urban Harare and selected rural communities in Zimbabwe. *J S Afr Vet Assoc,* 85, 1087.

CHITANGA, S., GAFF, H. & MUKARATIRWA, S. (2014) Tick-borne pathogens of potential zoonotic importance in the southern African Region. *J S Afr Vet Assoc,* 85, 1084.

CHIZEMA-KAWESHA, E., MILLER, J. M., STEKETEE, R. W., MUKONKA, V. M., MUKUKA, C., MOHAMED, A. D., MITI, S. K. & CAMPBELL, C. C. (2010) Scaling up malaria control in Zambia: progress and impact 2005â€“2008. *The American journal of tropical medicine and hygiene,* 83, 480-488.

CHOMEL, B. (2011) Tick-borne infections in dogs-an emerging infectious threat. *Vet Parasitol,* 179, 294-301.

CHOUDHURY, A. & MISRA, K. K. (1972) Experimental infection of T. evansi in the cat. *Trans R Soc Trop Med Hyg,* 66, 672-3.

CHOUDHURY, A. & MISRA, K. K. (1973) Letter: Occurrence of amastigote and sphaeromastigote stages of T. evansi in the brain tissue of the cat. *Trans R Soc Trop Med Hyg,* 67, 609.

CHOWDHURY, A. M. R., BHUIYA, A., CHOWDHURY, M. E., RASHEED, S., HUSSAIN, Z. & CHEN, L. C. (2013) The Bangladesh paradox: exceptional health achievement despite economic poverty. *The Lancet,* 382, 1734-1745.

CHRISTOPH, B. (2010) The relation between life satisfaction and the material situation: A re-evaluation using alternative measures. *Social Indicators Research,* 98, 475-499.

CHRISTOVA, I., VAN DE POL, J., YAZAR, S., VELO, E. & SCHOULS, L. (2003) Identification of Borrelia burgdorferi sensu lato, Anaplasma and Ehrlichia species, and spotted fever group Rickettsiae in ticks from Southeastern Europe. *Eur J Clin Microbiol Infect Dis,* 22, 535-42.

CITRO, C. F. & MICHAEL, R. T. (1995) Measuring Poverty. A New Approach, Panel on Poverty and Family Assistance: Concepts, Information Needs, and Measurement Methods. *National Research Council. Available online from http://www. nap. edu/catalog/4759. html.*

CLARIDGE, J., DIGGLE, P., MCCANN, C. M., MULCAHY, G., FLYNN, R., MCNAIR, J., STRAIN, S., WELSH, M., BAYLIS, M. & WILLIAMS, D. J. (2012) Fasciola hepatica is associated with the failure to detect bovine tuberculosis in dairy cattle. *Nat Commun,* 3, 853.

CLAUSEN, P. H., ADEYEMI, I., BAUER, B., BRELOEER, M., SALCHOW, F. & STAAK, C. (1998) Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. *Med Vet Entomol,* 12, 169-80.

COETZER, J. A. W. & TUSTIN, R. C. (Eds.) (2004a) *Infectious Diseases of Livestock,* 3.

COETZER, J. A. W. & TUSTIN, R. C. (Eds.) (2004b) *Infectious Diseases of Livestock,* 2.

COHEN, S., LINE, S., MANUCK, S. B., RABIN, B. S., HEISE, E. R. & KAPLAN, J. R. (1997) Chronic social stress, social status, and susceptibility to upper respiratory infections in nonhuman primates. *Psychosomatic Medicine,* 59, 213-221.

COLE, S. M. & HOON, P. N. (2013) Piecework (ganyu) as an indicator of household vulnerability in rural Zambia. *Ecol Food Nutr,* 52, 407-26.

COLEMAN, M., COLEMAN, M., MABASO, M. L., MABUZA, A. M., KOK, G., COETZEE, M. & DURRHEIM, D. N. (2010) Household and microeconomic factors associated with malaria in Mpumalanga, South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 104, 143-147.

COOK, R. J. (1993) International human rights and women's reproductive health. *Studies in family planning*, 73-86.

COUTTS, C. & HAHN, M. (2015) Green infrastructure, ecosystem services, and human health. *International Journal of Environmental Research and Public Health,* 12, 9768-9798.

COX, A. P., TOSAS, O., TILLEY, A., PICOZZI, K., COLEMAN, P., HIDE, G. & WELBURN, S. C. (2010) Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. *Parasit Vectors,* 3, 82.

CRAIG, P. & PAWLOWSKI, Z. (2002) Taenia solium cysticercosis in Africa: an under-recognised problem. *Cestode Zoonoses: Echinococcosis and Cysticercosis: An Emergent and Global Problem,* 341, 13.

CRISTOBAL-AZKARATE, J., DUNN, J. C., DAY, J. M. & AMABILE-CUEVAS, C. F. (2014) Resistance to antibiotics of clinical relevance in the fecal microbiota of Mexican wildlife. *PLoS One,* 9, e107719.

CROMPTON, D. W. T. (2006) *Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers*, World Health Organization

CROWELL, V., YUKICH, J. O., BRIET, O. J., ROSS, A. & SMITH, T. A. (2013) A novel approach for measuring the burden of uncomplicated Plasmodium falciparum malaria: application to data from Zambia. *PLoS One,* 8, e57297.

CUNNINGHAM, A. A., DASZAK, P. & WOOD, J. L. (2017) One Health, emerging infectious diseases and wildlife: two decades of progress? *Phil. Trans. R. Soc. B,* 372, 20160167.

D'ACCI, L. (2011) Measuring well-being and progress. *Social Indicators Research,* 104, 47-65.

DALAL-CLAYTON, B. & CHILD, B. (2003) Lessons from Luangwa:The Story of the Luangwa Integrated Resource Development Project, Zambia, March

DALAL-CLAYTON, B., DALAL-CLAYTON, D. B. & CHILD, B. (2003) *Lessons from Luangwa: The Story of the Luangwa Integrated Resource Development Project, Zambia*, IIED

DANTAS-TORRES, F., CHOMEL, B. B. & OTRANTO, D. (2012) Ticks and tick-borne diseases: a One Health perspective. *Trends in parasitology,* 28, 437-446.

DE GLANVILLE, W. A., CONDE-ÁLVAREZ, R., MORIYÓN, I., NJERU, J., DÍAZ, R., COOK, E. A. J., MORIN, M., BRONSVOORT, B. M. C., THOMAS, L. F., KARIUKI, S. & FÈVRE, E. M. (2017) Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLoS Negl Trop Dis,* 11.

DEBORGGRAEVE, S., LEJON, V., EKANGU, R. A., NGOYI, D. M., PYANA, P. P., ILUNGA, M. D., MULUNDA, J. P. & BÃ¼SCHER, P. (2011) Diagnostic accuracy of PCR in gambiense sleeping sickness diagnosis, staging and post-treatment follow-up: a 2-year longitudinal study. *PLOS Neglected Tropical Diseases,* 5, e972.

DEL BRUTTO, O. H. (2012) Diagnostic criteria for neurocysticercosis, revisited. *Pathog Glob Health,* 106, 299-304.

DELESPAUX, V., DINKA, H., MASUMU, J., VAN DEN BOSSCHE, P. & GEERTS, S. (2008) Five-fold increase in Trypanosoma congolense isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. *Drug Resistance Updates,* 11, 205-209.

DESQUESNES, M. & DIA, M. L. (2003) Mechanical transmission of Trypanosoma congolense in cattle by the African tabanid Atylotus agrestis. *Exp Parasitol,* 105, 226-31.

DIAZ-APARICIO, E., MARIN, C., ALONSO-URMENETA, B., ARAGON, V., PEREZ-ORTIZ, S., PARDO, M., BLASCO, J. M., DIAZ, R. & MORIYON, I. (1994) Evaluation of serological tests for diagnosis of Brucella melitensis infection of goats. *J Clin Microbiol,* 32, 1159-65.

DIAZ, R., CASANOVA, A., ARIZA, J. & MORIYON, I. (2011) The Rose Bengal Test in human brucellosis: a neglected test for the diagnosis of a neglected disease. *PLoS Negl Trop Dis,* 5, e950.

DOLAN, P., PEASGOOD, T. & WHITE, M. (2008) Do we really know what makes us happy? A review of the economic literature on the factors associated with subjective well-being. *Journal of economic psychology,* 29, 94-122.

DORAN, M. (2000) *Socio-economics of trypanosomosis*, RTTCP, Harare, Zimbabwe 3.

DORNY, P., BRANDT, J., ZOLI, A. & GEERTS, S. (2003) Immunodiagnostic tools for human and porcine cysticercosis. *Acta Trop,* 87, 79-86.

DORNY, P., PHIRI, I., GABRIEL, S., SPEYBROECK, N. & VERCRUYSSE, J. (2002) A sero-epidemiological study of bovine cysticercosis in Zambia. *Vet Parasitol,* 104, 211-5.

DORNY, P., PHIRI, I. K., VERCRUYSSE, J., GABRIEL, S., WILLINGHAM, A. L., 3RD, BRANDT, J., VICTOR, B., SPEYBROECK, N. & BERKVENS, D. (2004) A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis. *Int J Parasitol,* 34, 569-76.

DUCHEYNE, E., MWEEMPWA, C., DE PUS, C., VERNIEUWE, H., DE DEKEN, R., HENDRICKX, G. & VAN DEN BOSSCHE, P. (2009) The impact of habitat fragmentation on tsetse abundance on the plateau of eastern Zambia. *Prev Vet Med,* 91, 11-8.

DUCROTOY, M., BERTU, W. J., MATOPE, G., CADMUS, S., CONDE-ALVAREZ, R., GUSI, A. M., WELBURN, S., OCHOLI, R., BLASCO, J. M. & MORIYON, I. (2015) Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Trop*.

DUCROTOY, M. J., CONDE-Ã□LVAREZ, R., BLASCO, J. M. A. & MORIYÃ³N, I. (2016) A review of the basis of the immunological diagnosis of ruminant brucellosis. *Veterinary Immunology and Immunopathology,* 171, 81-102.

DUKES, P., SCOTT, C., RICKMAN, L. & WUPARA, F. (1983) Sleeping sickness in the Luangwa Valley of Zambia. A preliminary report of the 1982 outbreak at Kasyasya village. *Bulletin de la Societe de pathologie exotique et de ses filiales,* 76, 605-613.

EISELE, T. P., LARSEN, D. A., ANGLEWICZ, P. A., KEATING, J., YUKICH, J., BENNETT, A., HUTCHINSON, P. & STEKETEE, R. W. (2012) Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. *The Lancet infectious diseases,* 12, 942-949.

EJF (2017) *The deadly chemicals in cotton*, Environmental Justice Foundation in collaboration with Pesticide Action Network UK, London, UK.

ELHAMIANI KHATAT, S., SAHIBI, H., HING, M., ALAOUI MOUSTAIN, I., EL AMRI, H., BENAJIBA, M., KACHANI, M., DUCHATEAU, L. & DAMINET, S.

(2016) Human Exposure to Anaplasma phagocytophilum in Two Cities of Northwestern Morocco. *PLoS One,* 11, e0160880.

ENSOR, T., GREEN, C., QUIGLEY, P., BADRU, A. R., KALUBA, D. & KUREYA, T. (2014) Mobilizing communities to improve maternal health: results of an intervention in rural Zambia. *Bull World Health Organ,* 92, 51-9.

EVANS, P. (1996) Government action, social capital and development: reviewing the evidence on synergy. *World development,* 24, 1119-1132.

EYGELAAR, D., JORI, F., MOKOPASETSO, M., SIBEKO, K. P., COLLINS, N. E., VORSTER, I., TROSKIE, M. & OOSTHUIZEN, M. C. (2015) Tick-borne haemoparasites in African buffalo (Syncerus caffer) from two wildlife areas in Northern Botswana. *Parasit Vectors,* 8, 26.

FABURAY, B. (2015) The case for a 'one health' approach to combating vector-borne diseases. *Infection ecology & epidemiology,* 5, 28132.

FAO. Porcine brucellosis [online]. Available from: http://www.fao.org/ag/againfo/programmes/en/empres/gemp/avis/b103-brucellosis/mod0/0320-diagnosis-indivdual.html

FAO. Use of Pour-on Technology to Control Ectoparasites [online]. Available from: http://www.fao.org/ag/againfo/programmes/en/lead/toolbox/Grazing/Pour_on.htm [6 August 2015]

FAO. (2012) Thoughts of FAO on 'One Health' [online]. Available from: http://www.fao.org/ag/againfo/home/en/news_archive/2010_one-health.html

FAO, OIE & WHO COLLABORATION (2010) Tripartite Concept Note: sharing responsibilities and coordinating global activities to address health risks at the animal-human-ecosystems interfaces. *World Health Organization. http://www.oie.int/fileadmin/Home/eng/Current_Scientific_Issues/docs/pdf/FINAL_CONCEPT_NOTE_Hanoi.pdf (last accessed 2013).*

FAO, WHO, OIE, UNSIC, UNICEF & WB (2008) Contributing to one world, one health: a strategic framework for reducing risks of infectious diseases at the animal-human-ecosystems interface. *Consultation document*.

FAO REGIONAL OFFICE FOR AFRICA (2014) Closing the livestock gender gap in Southern Africa.

FEASEY, N., WANSBROUGH-JONES, M., MABEY, D. C. & SOLOMON, A. W. (2010) Neglected tropical diseases. *Br Med Bull,* 93, 179-200.

FERGUSON, H. M., DORNHAUS, A., BEECHE, A., BORGEMEISTER, C., GOTTLIEB, M., MULLA, M. S., GIMNIG, J. E., FISH, D. & KILLEEN, G. F. (2010) Ecology: a prerequisite for malaria elimination and eradication. *PLoS medicine,* 7, e1000303.

FÈVRE, E. M., WISSMANN, B. V., WELBURN, S. C. & LUTUMBA, P. (2008) The burden of human African trypanosomiasis. *PLoS Negl Trop Dis,* 2, e333.

FORSGREN, L. (2008) Estimations of the prevalence of epilepsy in sub-Saharan Africa. *Lancet Neurol,* 7, 21-2.

FOX-RUSHBY, J. & CAIRNS, J. (2005) *Economic evaluation*, McGraw-Hill Education (UK)

FRANCO, A. O., GOMES, M. G., ROWLAND, M., COLEMAN, P. G. & DAVIES, C. R. (2014) Controlling malaria using livestock-based interventions: a one health approach. *PLoS One,* 9, e101699.

FREDERICK (2013) Aerial Survey Report: Luangwa Valley 2012, COMACO, Lusaka.

FREEMAN, H. A. (2008) *Livestock, Livelihoods, and Vulnerability in Lesotho, Malawi, and Zambia: Designing Livestock Interventions for Emergency Situations*, ILRI (aka ILCA and ILRAD) 8.

FUNK, S., NISHIURA, H., HEESTERBEEK, H., EDMUNDS, W. J. & CHECCHI, F. (2013) Identifying transmission cycles at the human-animal interface: the role of animal reservoirs in maintaining gambiense human african trypanosomiasis. *PLoS computational biology,* 9, e1002855.

GABRIEL, S., BLOCHER, J., DORNY, P., ABATIH, E. N., SCHMUTZHARD, E., OMBAY, M., MATHIAS, B. & WINKLER, A. S. (2012) Added value of antigen ELISA in the diagnosis of neurocysticercosis in resource poor settings. *PLoS Negl Trop Dis,* 6, e1851.

GALAZ, V., LEACH, M., SCOONES, I. & STEIN, C. (2015) The political economy of One Health research and policy.

GALLAGHER, J. (1973) The rose bengal plate agglutination test in dairy cattle in Zambia vaccinated over age with strain 19 Brucella abortus. *Trop Anim Health Prod,* 5, 253-8.

GAO, Y. & CLARK, S. G. (2014) Elephant ivory trade in China: Trends and drivers. *Biological conservation,* 180, 23-30.

GEORGES, K., LORIA, G. R., RIILI, S., GRECO, A., CARACAPPA, S., JONGEJAN, F. & SPARAGANO, O. (2001) Detection of haemoparasites in cattle by reverse line blot hybridisation with a note on the distribution of ticks in Sicily. *Vet Parasitol,* 99, 273-86.

GHIROTTI, M., SEMPRONI, G., DE MENEGHI, D., MUNGABA, F. N., NANNINI, D., CALZETTA, G. & PAGANICO, G. (1991) Sero-prevalences of selected cattle diseases in the Kafue flats of Zambia. *Vet Res Commun,* 15, 25-36.

GIBSON, W. C., STEVENS, J. R., MWENDIA, C. M., NGOTHO, J. N. & NDUNG'U, J. M. (2001) Unravelling the phylogenetic relationships of African trypanosomes of suids. *Parasitology,* 122, 625-31.

GILLET, P., NGOYI, D. M., LUKUKA, A., KANDE, V., ATUA, B., VAN GRIENSVEN, J., MUYEMBE, J.-J., JACOBS, J. & LEJON, V. (2013) False positivity of non-targeted infections in malaria rapid diagnostic tests: the case of human african trypanosomiasis. *PLOS Neglected Tropical Diseases,* 7, e2180.

GLYNN, P. W. (1993) Coral reef bleaching: ecological perspectives. *Coral Reefs,* 12, 1-17.

GODFROID, J. (2002) Brucellosis in wildlife. *Revue Scientifique et Technique-Office international des eÌ□pizooties,* 21, 277-286.

GORDON, G. & MWALE, V. (2006) Preventing HIV with young people: a case study from Zambia. *Reproductive Health Matters,* 14, 68-79.

GOVENDER, D., OOSTHUISEN, M. & PENZHORN, B. L. (2011a) Piroplasm parasites of white rhinoceroses (Ceratotherium simum) in the Kruger National Park, and their relation to anaemia. *Journal of the South African Veterinary Association,* 82, 36-40.

GOVENDER, D., OOSTHUIZEN, M. C. & PENZHORN, B. L. (2011b) Piroplasm parasites of white rhinoceroses (Ceratotherium simum) in the Kruger National Park, and their relation to anaemia. *J S Afr Vet Assoc,* 82, 36-40.

GRACE, D., GILBERT, J., RANDOLPH, T. & KANG'ETHE, E. (2012) The multiple burdens of zoonotic disease and an Ecohealth approach to their assessment. *Trop Anim Health Prod,* 44 Suppl 1, S67-73.

GRAHAM, H. (2004) Social determinants and their unequal distribution: clarifying policy understandings. *The Milbank Quarterly,* 82, 101-124.

GRANDIN, B. E. (1988) *Wealth ranking in smallholder communities: a field manual*, Intermediate Technology Publications

GUBBELS, J. M., DE VOS, A. P., VAN DER WEIDE, M., VISERAS, J., SCHOULS, L. M., DE VRIES, E. & JONGEJAN, F. (1999) Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. *J Clin Microbiol,* 37, 1782-9.

GUMBO, D., MOOMBE, K. B., KABWE, G., OJANEN, M., NDHLOVU, E., SUNDERLAND, T. & KANDULU, M. (2013) Dynamics of the charcoal and indigenous timber trade in Zambia: A scoping study in Eastern, Northern and Northwestern provinces.

HADDAD, L., WALL, K. M., VWALIKA, B., KHU, N. H., BRILL, I., KILEMBE, W., STEPHENSON, R., CHOMBA, E., VWALIKA, C. & TICHACEK, A. (2013)

Contraceptive discontinuation and switching among couples receiving integrated HIV and family planning services in Lusaka, Zambia. *AIDS (London, England),* 27, S93.

HALL, V., THOMSEN, R. W., HENRIKSEN, O. & LOHSE, N. (2011) Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications. A systematic review. *BMC Public Health,* 11, 564.

HALLIDAY, J., DABORN, C., AUTY, H., MTEMA, Z., LEMBO, T., BAREND, M., HANDEL, I., KNOBEL, D., HAMPSON, K. & CLEAVELAND, S. (2012) Bringing together emerging and endemic zoonoses surveillance: shared challenges and a common solution. *Phil. Trans. R. Soc. B,* 367, 2872-2880.

HARRISON, R. E., PEARSON, L., VERE, M., CHONZI, P., HOVE, B. T., MABAYA, S., CHIGWAMBA, M., NHAMBURO, J., GURA, J. & VANDEBORNE, A. (2017) Care requirements for clients who present after rape and clients who presented after consensual sex as a minor at a clinic in Harare, Zimbabwe, from 2011 to 2014. *PLoS One,* 12, e0184634.

HARVEY, K., ESPOSITO, D. H., HAN, P., KOZARSKY, P., FREEDMAN, D. O., PLIER, D. A. & SOTIR, M. J. (2013) Surveillance for travel-related diseaseâ€"GeoSentinel surveillance system, United States, 1997â€"2011. *Morbidity and Mortality Weekly Report: Surveillance Summaries,* 62, 1-23.

HOLMES, P. H., KATUNGUKA-RWAKISHAYA, E., BENNISON, J. J., WASSINK, G. J. & PARKINS, J. J. (2000) Impact of nutrition on the pathophysiology of bovine trypanosomiasis. *Parasitology,* 120 Suppl, S73-85.

HOPKINS, A. D. (2016) Neglected tropical diseases in Africa: a new paradigm. *Int Health,* 8 Suppl 1, i28-33.

HORTON, R. & LO, S. (2015) Planetary health: a new science for exceptional action. *The Lancet*.

HOTEZ, P. J. & KAMATH, A. (2009) Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl Trop Dis,* 3, e412.

HOUGH, R. L. (2014) Biodiversity and human health: evidence for causality? *Biodiversity and conservation,* 23, 267-288.

HOWE, K. (1985) Economist's view of animal disease. *Society for Veterinary Epidemiology and Preventive Medicine: proceedings of a meeting held at the University of Reading on the 27th, 28th, and 29th of March 1985/edited by MV Thrusfield.* [Edinburgh]: The Society, c1985.

HUBER, J. D. & NICOLETTI, P. (1986) Comparison of the results of card, rivanol, complement-fixation, and milk ring tests with the isolation rate of Brucella abortus from cattle. *Am J Vet Res,* 47, 1529-31.

HUDSON, P. J., DOBSON, A. P. & LAFFERTY, K. D. (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution,* 21, 381-385.

HYDER, A. A., ROTLLANT, G. & MORROW, R. H. (1998) Measuring the burden of disease: healthy life-years. *American Journal of Public Health,* 88, 196-202.

JALETA, K. T., HILL, S. R., BIRGERSSON, G. R., TEKIE, H. & IGNELL, R. (2016) Chicken volatiles repel host-seeking malaria mosquitoes. *Malaria journal,* 15, 354.

JIWA, S. F., KAZWALA, R. R., TUNGARAZA, R., KIMERA, S. I. & KALAYE, W. J. (1996) Bovine brucellosis serum agglutination test prevalence and breed disposition according to prevalent management systems in the Lake Victoria zone of Tanzania. *Preventive Veterinary Medicine,* 26, 341-346.

JOHN, K., KAZWALA, R. & MFINANGA, G. S. (2008) Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC infectious diseases,* 8, 162.

JONES, B. A., GRACE, D., KOCK, R., ALONSO, S., RUSHTON, J., SAID, M. Y., MCKEEVER, D., MUTUA, F., YOUNG, J. & MCDERMOTT, J. (2013) Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences,* 110, 8399-8404.

JONES, K. E., PATEL, N. G., LEVY, M. A., STOREYGARD, A., BALK, D., GITTLEMAN, J. L. & DASZAK, P. (2008) Global trends in emerging infectious diseases. *Nature,* 451, 990-3.

JONGEJAN, F., MUSISI, F. L., MOORHOUSE, P. D., SNACKEN, M. & UILENBERG, G. (1986) Theileria taurotragi in Zambia. *Vet Q,* 8, 261-3.

JONSSON, N. N., BOCK, R. E., JORGENSEN, W. K., MORTON, J. M. & STEAR, M. J. (2012) Is endemic stability of tick-borne disease in cattle a useful concept? *Trends Parasitol,* 28, 85-9.

KAARE, M. & CLEAVELAND, S. (1997) Targeting rabies control in the Serengeti: the rationale and design of a domestic dog vaccination campaign. *Proceedings of the Fourth International Conference of the Southern and Eastern African Rabies Group. Nairobi, Kenya.*

KALYOCHA G.C.K. (1988) Luangwa Integrated Resource Development Project - Detailed socio-economic survey of the LIRDP area,

KAMANGA, A., MOONO, P., STRESMAN, G., MHARAKURWA, S. & SHIFF, C. (2010) Rural health centres, communities and malaria case detection in Zambia using mobile telephones: a means to detect potential reservoirs of infection in unstable transmission conditions. *Malaria journal,* 9, 96.

KAMANI, T. M., KAZWALA, R., MFINANGA, S., HAYDON, D., KEYYU, J., LANKESTER, F. & BUZA, J. (2015) One Health: a concept led by Africa, with global benefits. *The Veterinary record,* 176, 496.

KAMULIWO, M., CHANDA, E., HAQUE, U., MWANZA-INGWE, M., SIKAALA, C., KATEBE-SAKALA, C., MUKONKA, V. M., NORRIS, D. E., SMITH, D. L. & GLASS, G. E. (2013) The changing burden of malaria and association with vector control interventions in Zambia using district-level surveillance data, 2006-2011. *Malaria journal,* 12, 437.

KEATING, J., MILLER, J. M., BENNETT, A., MOONGA, H. B. & EISELE, T. P. (2009) Plasmodium falciparum parasite infection prevalence from a household survey in Zambia using microscopy and a rapid diagnostic test: implications for monitoring and evaluation. *Acta tropica,* 112, 277-282.

KEESING, F., BELDEN, L. K., DASZAK, P., DOBSON, A., HARVELL, C. D., HOLT, R. D., HUDSON, P., JOLLES, A., JONES, K. E. & MITCHELL, C. E. (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature,* 468, 647-652.

KEESING, F., HOLT, R. D. & OSTFELD, R. S. (2006) Effects of species diversity on disease risk. *Ecology Letters,* 9, 485-498.

KENIGER, L. E., GASTON, K. J., IRVINE, K. N. & FULLER, R. A. (2013) What are the benefits of interacting with nature? *International Journal of Environmental Research and Public Health,* 10, 913-935.

KENNEDY, P. G. (2013) Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). *The Lancet Neurology,* 12, 186-194.

KITALA, P., MCDERMOTT, J., KYULE, M., GATHUMA, J., PERRY, B. & WANDELER, A. (2001) Dog ecology and demography information to support the planning of rabies control in Machakos District, Kenya. *Acta tropica,* 78, 217-230.

KOCHER, T. D., THOMAS, W. K., MEYER, A., EDWARDS, S. V., PAABO, S., VILLABLANCA, F. X. & WILSON, A. C. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci U S A,* 86, 6196-200.

KONG, F. & GILBERT, G. L. (2006) Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB) - a practical epidemiological and diagnostic tool. *Nature protocols,* 1, 2668-2680.

KRISTJANSON, P. M., SWALLOW, B. M., ROWLANDS, G., KRUSKA, R. & DE LEEUW, P. (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems,* 59, 79-98.

KUNDA, J., FITZPATRICK, J., KAZWALA, R., FRENCH, N. P., SHIRIMA, G., MACMILLAN, A., KAMBARAGE, D., BRONSVOORT, M. & CLEAVELAND,

S. (2007) Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health,* 7, 315.

KUNZ, S. & KEMP, D. (1994) Insecticides and acaricides: resistance and environmental impact. *Revue scientifique et technique (International Office of Epizootics),* 13, 1249-1286.

KWARTENG, A., AHUNO, S. T. & AKOTO, F. O. (2016) Killing filarial nematode parasites: role of treatment options and host immune response. *Infect Dis Poverty,* 5, 86.

LARA, R., ISLAM, M., YAMASAKI, S., NEOGI, S. & NAIR, G. (2011) 10.12 Aquatic Ecosystems, Human Health, and Ecohydrology.

LEEMANS, R. & DE GROOT, R. (2003) *Millennium Ecosystem Assessment: Ecosystems and human well-being: a framework for assessment*, Island Press

LEOPOLD, A. (1989) *A Sand County almanac, and sketches here and there*, Outdoor Essays & Reflections

LIM, M.-K. (2004) Shifting the burden of health care finance: a case study of public-private partnership in Singapore. *Health policy,* 69, 83-92.

LISULO, M., SUGIMOTO, C., KAJINO, K., HAYASHIDA, K., MUDENDA, M., MOONGA, L., NDEBE, J., NZALA, S. & NAMANGALA, B. (2014) Determination of the prevalence of African trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by loop-mediated isothermal amplification. *Parasit Vectors,* 7, 19.

LOBO, N. F., LAURENT, B. S., SIKAALA, C. H., HAMAINZA, B., CHANDA, J., CHINULA, D., KRISHNANKUTTY, S. M., MUELLER, J. D., DEASON, N. A. & HOANG, Q. T. (2015) Unexpected diversity of Anopheles species in Eastern Zambia: implications for evaluating vector behavior and interventions using molecular tools. *Scientific Reports,* 5.

LORENZ, M. O. C. F. P. D. J. (1905) Methods of Measuring the Concentration of Wealth. *Publications of the American Statistical Association,* 9, 209-219.

LORUSSO, V. (2014) Epidemiology and Control of cattle ticks and tick-borne infections in Central Nigeria. University of Edinburgh.

MACHILA, N., SINYANGWE, L., MUBANGA, J., HOPKINS, J. S., ROBINSON, T. & EISLER, M. C. (2001) Antibody-ELISA seroprevalence of bovine trypanosomosis in the Eastern Province of Zambia. *Prev Vet Med,* 49, 249-57.

MACLEAN, L. M., ODIIT, M., CHISI, J. E., KENNEDY, P. G. & STERNBERG, J. M. (2010) Focus-specific clinical profiles in human African trypanosomiasis caused by Trypanosoma brucei rhodesiense. *PLOS Neglected Tropical Diseases,* 4, e906.

MAFOJANE, N. A., APPLETON, C. C., KRECEK, R. C., MICHAEL, L. M. & WILLINGHAM, A. L., 3RD (2003) The current status of neurocysticercosis in Eastern and Southern Africa. *Acta Trop,* 87, 25-33.

MAKALA, L. H., MANGANI, P., FUJISAKI, K. & NAGASAWA, H. (2003) The current status of major tick borne diseases in Zambia. *Vet Res,* 34, 27-45.

MALAMA, S., MUMA, J. B. & GODFROID, J. (2013) A review of tuberculosis at the wildlife-livestock-human interface in Zambia. *Infect Dis Poverty,* 2, 13.

MALLEWA, M., FOOKS, A. R., BANDA, D., CHIKUNGWA, P., MANKHAMBO, L., MOLYNEUX, E., MOLYNEUX, M. E. & SOLOMON, T. (2007) Rabies encephalitis in malaria-endemic area, Malawi, Africa. *Emerging infectious diseases,* 13, 136.

MANASE, G., MULENGA, M. & FAWCETT, B. (2001) Linking urban sanitation agencies with poor community needs: a study of Zambia, Zimbabwe and South Africa. *London: DFID Knowledge and Research Project. Available on the Internet at: http://www. eng4dev. soton. ac. uk/eng4devpdfs,* 7124.

MARCOGLIESE, D. J. (2005) Parasites of the superorganism: are they indicators of ecosystem health? *International journal for parasitology,* 35, 705-716.

MARKS, S. & FULLER, R. (2008) Enclosure of an important wildlife commons in Zambia. *International Association for the Study of the Commons conference at University of Gloucestershire, Cheltenham, UK.*

MARKS, S. A. (1976) *Large mammals and a brave people: Subsistence hunters in Zambia*, Transaction Publishers

MARTINS, T. M., NEVES, L., PEDRO, O. C., FAFETINE, J. M., DO ROSARIO, V. E. & DOMINGOS, A. (2010) Molecular detection of Babesia spp. and other haemoparasitic infections of cattle in Maputo Province, Mozambique. *Parasitology,* 137, 939-946.

MASIGA, D. K., MCNAMARA, J. J. & GIBSON, W. C. (1996) A repetitive DNA sequence specific for Trypanosoma (Nannomonas) godfreyi. *Vet Parasitol,* 62, 27-33.

MASUMU, J., MARCOTTY, T., GEYSEN, D., GEERTS, S., VERCRUYSSE, J., DORNY, P. & VAN DEN BOSSCHE, P. (2006a) Comparison of the virulence of Trypanosoma congolense strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *International journal for parasitology,* 36, 497-501.

MASUMU, J., MARCOTTY, T., NDELEDJE, N., KUBI, C., GEERTS, S., VERCRUYSSE, J., DORNY, P. & VAN DEN BOSSCHE, P. (2006b) Comparison of the transmissibility of Trypanosoma congolense strains, isolated in a trypanosomiasis endemic area of eastern Zambia, by Glossina morsitans morsitans. *Parasitology,* 133, 331-334.

MASUMU, J., TSHILENGE, G. & MBAO, V. (2012) *Epidemiological aspects of bovine trypanosomosis in an endemic focus of eastern Zambia: The role of trypanosome strain variability in disease pattern,* 79.

MATEMBA, L. E., FÈVRE, E. M., KIBONA, S. N., PICOZZI, K., CLEAVELAND, S., SHAW, A. P. & WELBURN, S. C. (2010) Quantifying the burden of rhodesiense sleeping sickness in Urambo District, Tanzania. *PLoS Negl Trop Dis,* 4, e868.

MATETE, G. O. (2003) Occurrence, clinical manifestation and the epidemiological implications of naturally occurring canine trypanosomosis in western Kenya. *Onderstepoort J Vet Res,* 70, 317-23.

MATHERS, C. (2008) *The global burden of disease: 2004 update*, World Health Organization

MATJILA, P. T., LEISEWITZ, A. L., JONGEJAN, F. & PENZHORN, B. L. (2008) Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. *Vet Parasitol,* 155, 152-7.

MAXWELL, L., DEVRIES, K., ZIONTS, D., ALHUSEN, J. L. & CAMPBELL, J. (2015) Estimating the effect of intimate partner violence on women's use of contraception: a systematic review and meta-analysis. *PLoS One,* 10, e0118234.

MAYAGAYA, V. S., NKWENGULILA, G., LYIMO, I. N., KIHONDA, J., MTAMBALA, H., NGONYANI, H., RUSSELL, T. L. & FERGUSON, H. M. (2015) The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. *Malar J,* 14, 17.

MAZET, J. A., CLIFFORD, D. L., COPPOLILLO, P. B., DEOLALIKAR, A. B., ERICKSON, J. D. & KAZWALA, R. R. (2009) A 'One Health' approach to address emerging zoonoses: the HALI project in Tanzania. *PLoS Med,* 6, e1000190.

MBUGI, E. V., KAYUNZE, K. A., KATALE, B. Z., KENDALL, S., GOOD, L., KIBIK, G. S., KEYYU, J. D., GODFREY-FAUSSETT, P., VAN HELDEN, P. & MATEE, M. I. (2012) 'One Health' infectious diseases surveillance in Tanzania: Are we all on board the same flight? *Onderstepoort Journal of Veterinary Research,* 79, 01-07.

MCNAMARA, J. J., MOHAMMED, G. & GIBSON, W. C. (1994) Trypanosoma (Nannomonas) godfreyi sp. nov. from tsetse flies in The Gambia: biological and biochemical characterization. *Parasitology,* 109 (Pt 4), 497-509.

MELLANBY, R., HANDEL, I., CLEMENTS, D., C BRONSVOORT, B., LENGELING, A. & SCHOEMAN, J. (2011) Breed and sex risk factors for canine babesiosis in South Africa. *Journal of Veterinary Internal Medicine,* 25, 1186-1189.

MINJAUW, B. & MCLEOD, A. (2003) Tick-borne diseases and poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. *Tick-borne diseases and* *poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa.*

MISHAN, E. J. (1971) Evaluation of life and limb: a theoretical approach. *Journal of Political Economy,* 79, 687-705.

MOLYNEUX, D., HALLAJ, Z., KEUSCH, G. T., MCMANUS, D. P., NGOWI, H., CLEAVELAND, S., RAMOS-JIMENEZ, P., GOTUZZO, E., KAR, K. & SANCHEZ, A. (2011) Zoonoses and marginalised infectious diseases of poverty: where do we stand? *Parasites & vectors,* 4, 106.

MONATH, T. P. (2013) Vaccines against diseases transmitted from animals to humans: a one health paradigm. *Vaccine,* 31, 5321-38.

MOSER, D. R., COOK, G. A., OCHS, D. E., BAILEY, C. P., MCKANE, M. R. & DONELSON, J. E. (1989) Detection of Trypanosoma congolense and Trypanosoma brucei subspecies by DNA amplification using the polymerase chain reaction. *Parasitology,* 99, 57-66.

MUBAMBA, C., SITALI, J. & GUMMOW, B. (2011) Trends of selected cattle diseases in eastern Zambia between 1988 and 2008. *Prev Vet Med,* 101, 163-72.

MUBANGA, J. (2008) Animal trypanosomiasis in the Eastern Province of Zambia: epidemiology in the recently-settled areas and evaluation of a novel method for control. University of Edinburgh.

MUMA, J. B., GODFROID, J., SAMUI, K. L. & SKJERVE, E. (2007a) The role of Brucella infection in abortions among traditional cattle reared in proximity to wildlife on the Kafue flats of Zambia. *Rev Sci Tech,* 26, 721-30.

MUMA, J. B., LUND, A., NIELSEN, K., MATOPE, G., MUNYEME, M., MWACALIMBA, K. & SKJERVE, E. (2009) Effectiveness of Rose Bengal test and fluorescence polarization assay in the diagnosis of Brucella spp. infections in free range cattle reared in endemic areas in Zambia. *Trop Anim Health Prod,* 41, 723-9.

MUMA, J. B., LUND, A., SIAMUDAALA, V. M., MUNANG'ANDU, H. M., MUNYEME, M., MATOPE, G., NIELSEN, K., B, D. J., GODFROID, J., TRYLAND, M. & SKJERVE, E. (2010) Serosurvey of Brucella spp. infection in the Kafue lechwe (Kobus leche kafuensis) of the Kafue flats in Zambia. *J Wildl Dis,* 46, 1063-9.

MUMA, J. B., MUNYEME, M., MATOPE, G., SIAMUDAALA, V. M., MUNANG'ANDU, H. M., MATANDIKO, W., GODFROID, J., SKJERVE, E. & TRYLAND, M. (2011) Brucella seroprevalence of the Kafue lechwe (Kobus leche kafuensis) and Black lechwe (Kobus leche smithemani): exposure associated to contact with cattle. *Prev Vet Med,* 100, 256-60.

MUMA, J. B., PANDEY, G. S., MUNYEME, M., MUMBA, C., MKANDAWIRE, E. & CHIMANA, H. M. (2012) Brucellosis among smallholder cattle farmers in Zambia: public health significance. *Trop Anim Health Prod,* 44, 915-20.

MUMA, J. B., SAMUI, K. L., OLOYA, J., MUNYEME, M. & SKJERVE, E. (2007b) Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Prev Vet Med,* 80, 306-17.

MUMA, J. B., SAMUI, K. L., SIAMUDAALA, V. M., OLOYA, J., MATOP, G., OMER, M. K., MUNYEME, M., MUBITA, C. & SKJERVE, E. (2006) Prevalence of antibodies to Brucella spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. *Trop Anim Health Prod,* 38, 195-206.

MUMA, J. B., SYAKALIMA, M., MUNYEME, M., ZULU, V. C., SIMUUNZA, M. & KURATA, M. (2013) Bovine tuberculosis and brucellosis in traditionally managed livestock in selected districts of southern province of zambia. *Vet Med Int,* 2013, 730367.

MUMA, J. B., TOFT, N., OLOYA, J., LUND, A., NIELSEN, K., SAMUI, K. & SKJERVE, E. (2007c) Evaluation of three serological tests for brucellosis in naturally infected cattle using latent class analysis. *Vet Microbiol,* 125, 187-92.

MUNANG'ANDU, H. M., MWEENE, A. S., SIAMUDAALA, V., MUMA, J. B. & MATANDIKO, W. (2011) Rabies status in Zambia for the period 1985-2004. *Zoonoses Public Health,* 58, 21-7.

MUNANG'ANDU, H. M., SIAMUDAALA, V., MATANDIKO, W., MULUMBA, M., NAMBOTA, A., MUNYEME, M., MUTOLOKI, S. & NONGA, H. (2009) Detection of Theileria parva antibodies in the African buffalo (Syncerus caffer) in the livestock-wildlife interface areas of Zambia. *Vet Parasitol,* 166, 163-6.

MUNANG'ANDU, H. M., SIAMUDAALA, V. M., NAMBOTA, A., BWALYA, J. M., MUNYEME, M., MWEENE, A. S., TAKADA, A. & KIDA, H. (2006) Disease constraints for utilization of the African buffalo (Syncerus caffer) on game ranches in Zambia. *Jpn J Vet Res,* 54, 3-13.

MURRAY, C. J. (1994) Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bull World Health Organ,* 72, 429-45.

MURRAY, C. J. & CHEN, L. C. (1992) Understanding morbidity change. *The Population and Development Review*, 481-503.

MURRAY, C. J. & LOPEZ, A. D. (1997) Regional patterns of disability-free life expectancy and disability-adjusted life expectancy: Global Burden of Disease Study. *The Lancet,* 349, 1347-1352.

MURRAY, C. J., ROSENFELD, L. C., LIM, S. S., ANDREWS, K. G., FOREMAN, K. J., HARING, D., FULLMAN, N., NAGHAVI, M., LOZANO, R. & LOPEZ, A.

D. (2012) Global malaria mortality between 1980 and 2010: a systematic analysis. *The Lancet,* 379, 413-431.

MUSISI, F. L., JONGEJAN, F. & PEGRAM, R. G. (1984) Isolation and transmission of Theileria mutans (Chisamba) in Zambia. *Res Vet Sci,* 36, 129-31.

MUTURI, C. N., OUMA, J. O., MALELE, II, NGURE, R. M., RUTTO, J. J., MITHOFER, K. M., ENYARU, J. & MASIGA, D. K. (2011) Tracking the feeding patterns of tsetse flies (Glossina genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS One,* 6, e17284.

MWANAKASALE, V. & SONGOLO, P. (2011) Disappearance of some human African trypanosomiasis transmission foci in Zambia in the absence of a tsetse fly and trypanosomiasis control program over a period of forty years. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 105, 167-172.

MWANAKASALE, V., SONGOLO, P., BABANIYI, O. & SIMARRO, P. (2014) Clinical presentation of human African trypanosomiasis in Zambia is linked to the existence of strains of Trypanosoma brucei rhodesiense with varied virulence: two case reports. *Journal of medical case reports,* 8, 53.

MWANAKASALE, V., SONGOLO, P. & DAKA, V. (2013) Challenges in the control of Human African Trypanosomiasis in the Mpika district of Zambia. *BMC research notes,* 6, 180.

MWAPE, K. E., PHIRI, I. K., PRAET, N., MUMA, J. B., ZULU, G., VAN DEN BOSSCHE, P., DE DEKEN, R., SPEYBROECK, N., DORNY, P. & GABRIEL, S. (2012) Taenia solium Infections in a rural area of Eastern Zambia-a community based study. *PLoS Negl Trop Dis,* 6, e1594.

MWAPE, K. E., PHIRI, I. K., PRAET, N., SPEYBROECK, N., MUMA, J. B., DORNY, P. & GABRIÃ«L, S. (2013a) The incidence of human cysticercosis in a rural community of Eastern Zambia. *PLOS Neglected Tropical Diseases,* 7, e2142.

MWAPE, K. E., PHIRI, I. K., PRAET, N., SPEYBROECK, N., MUMA, J. B., DORNY, P. & GABRIEL, S. (2013b) The incidence of human cysticercosis in a rural community of Eastern Zambia. *PLoS Negl Trop Dis,* 7, e2142.

NAKAYIMA, J., HAYASHIDA, K., NAKAO, R., ISHII, A., OGAWA, H., NAKAMURA, I., MOONGA, L., HANG'OMBE, B. M., MWEENE, A. S., THOMAS, Y., ORBA, Y., SAWA, H. & SUGIMOTO, C. (2014) Detection and characterization of zoonotic pathogens of free-ranging non-human primates from Zambia. *Parasit Vectors,* 7, 490.

NAMANGALA, B., HACHAAMBWA, L., KAJINO, K., MWEENE, A. S., HAYASHIDA, K., SIMUUNZA, M., SIMUKOKO, H., CHOONGO, K., CHANSA, P. & LAKHI, S. (2012) The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasites & vectors,* 5, 282.

NAMANGALA, B., OPARAOCHA, E., KAJINO, K., HAYASHIDA, K., MOONGA, L., INOUE, N., SUZUKI, Y. & SUGIMOTO, C. (2013) Preliminary investigation of trypanosomosis in exotic dog breeds from Zambia's Luangwa and Zambezi valleys using LAMP. *Am J Trop Med Hyg,* 89, 116-8.

NEW ECONOMICS FOUNDATION. (2016) Happy planet index (HPI) [online]. Available from: http://happyplanetindex.org/

NEWTON, P. N., TABERNERO, P., DWIVEDI, P., CULZONI, M. J., MONGE, M. A. E., SWAMIDOSS, I., MILDENHALL, D., GREEN, M. D., JÃ¤HNKE, R. & DE OLIVEIRA, M. D. S. (2014) Falsified medicines in Africa: all talk, no action.

NG'AYO, M. O., NJIRU, Z. K., KENYA, E. U., MULUVI, G. M., OSIR, E. O. & MASIGA, D. K. (2005) Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biol Dis,* 4, 5.

NGOMA, M. S., NALUBAMBA, M., KOWA, S., MINYOI, D., MUBANGA, J., MWANSA, J., MUSUKU, J., CHINTU, C., BHAT, G. & KAPAKALA, T. (2004) Congenital trypanosomiasis. *Journal of tropical pediatrics,* 50, 377-378.

NICOLETTI, P. (1992) An evaluation of serologic tests used to diagnose brucellosis in buffaloes (Bubalus bubalis). *Trop Anim Health Prod,* 24, 40-4.

NIJHOF, A. M., BODAAN, C., POSTIGO, M., NIEUWENHUIJS, H., OPSTEEGH, M., FRANSSEN, L., JEBBINK, F. & JONGEJAN, F. (2007) Ticks and associated pathogens collected from domestic animals in the Netherlands. *Vector Borne Zoonotic Dis,* 7, 585-95.

NIJHOF, A. M., PENZHORN, B. L., LYNEN, G., MOLLEL, J. O., MORKEL, P., BEKKER, C. P. & JONGEJAN, F. (2003) Babesia bicornis sp. nov. and Theileria bicornis sp. nov.: tick-borne parasites associated with mortality in the black rhinoceros (Diceros bicornis). *J Clin Microbiol,* 41, 2249-54.

NIJHOF, A. M., PILLAY, V., STEYL, J., PROZESKY, L., STOLTSZ, W. H., LAWRENCE, J. A., PENZHORN, B. L. & JONGEJAN, F. (2005) Molecular characterization of Theileria species associated with mortality in four species of African antelopes. *J Clin Microbiol,* 43, 5907-11.

NJIRU, Z. K., CONSTANTINE, C. C., GUYA, S., CROWTHER, J., KIRAGU, J. M., THOMPSON, R. C. A. & DAVILA, A. (2005) The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitology Research,* 95, 186-192.

NNADI, P. A., EZEH, I. O., KALU, K. C. & NGENE, A. A. (2010) The impact of dietary protein on the pathophysiology of porcine trypanosome infection. *Vet Parasitol,* 173, 193-9.

NORRIS, L. C., MAIN, B. J., LEE, Y., COLLIER, T. C., FOFANA, A., CORNEL, A. J. & LANZARO, G. C. (2015) Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proceedings of the National Academy of Sciences,* 112, 815-820.

NYIRENDA, S. S., HANG'OMBE, B. M., MACHANG'U, R., MWANZA, J. & KILONZO, B. S. (2017) Identification of Risk Factors Associated with Transmission of Plague Disease in Eastern Zambia. *Am J Trop Med Hyg,* 97, 826-830.

NYIRENDA, V., LIWENA, S. & KAUMBA CHAKA, H. (2008) Atlas of the national parks of Zambia. *New Horizon Printing Press. Lusaka, Zambia*.

O'CONNELL, R., CHISHINGA, N., KINYANDA, E., PATEL, V., AYLES, H., WEISS, H. A. & SEEDAT, S. (2013) Prevalence and correlates of alcohol dependence disorder among TB and HIV infected patients in Zambia. *PLoS One,* 8, e74406.

O'SULLIVAN, M., ZHOU, F., SINTCHENKO, V., KONG, F. & GILBERT, G. L. (2011) Multiplex PCR and reverse line blot hybridization assay (mPCR/RLB). *J Vis Exp,* 54, e2781.

OECD. (2012) Definition gross national product [online]. Available from: http://stats.oecd.org/glossary/detail.asp?ID=1176

OECD. (2016) Gini coefficient [online]. Available from: https://data.oecd.org/inequality/income-inequality.htm

OECD. (2017) Organisation for Economic Cooperation and Development (OECD) better lives dashboard

[online]. Available from: http://www.oecd.org/std/na/household-dashboard.htm

OIE (2012) Manual of diagnostic tests and vaccines for terrestrial animals. *Office International des Epizooties, Paris, France*.

OKELLO, A. L., GIBBS, E. P., VANDERSMISSEN, A. & WELBURN, S. C. (2011) One Health and the neglected zoonoses: turning rhetoric into reality. *Vet Rec,* 169, 281-5.

OOSTHUIZEN, M. C., ALLSOPP, B. A., TROSKIE, M., COLLINS, N. E. & PENZHORN, B. L. (2009) Identification of novel Babesia and Theileria species in South African giraffe (Giraffa camelopardalis, Linnaeus, 1758) and roan antelope (Hippotragus equinus, Desmarest 1804). *Vet Parasitol,* 163, 39-46.

OSTERKAMP, R. (2013) The basic income grant pilot project in Namibia: a critical assessment. *Basic income studies,* 8, 71-91.

OTTE, M. & CHILONDA, P. (2002) Cattle and small ruminant production systems in sub-Saharan Africa. A systematic review.

OURA, C. A., TAIT, A., ASIIMWE, B., LUBEGA, G. W. & WEIR, W. (2011) Theileria parva genetic diversity and haemoparasite prevalence in cattle and wildlife in and around Lake Mburo National Park in Uganda. *Parasitology Research,* 108, 1365-1374.

PAROLA, P. (2006) Rickettsioses in Sub-Saharan Africa. *Annals of the New York Academy of Sciences,* 1078, 42-47.

PEARCE, N. & DOUWES, J. (2013) Research at the interface between human and veterinary health. *Preventive Veterinary Medicine,* 111, 187-193.

PERMANENT MISSION OF THE KINGDOM OF BHUTAN. (2005) Gross national happiness (GNH) [online]. Available from: https://www.un.int/bhutan/bhutan/gross-national-happiness

PERRY, B., MWANAUMO, B., SCHELS, H., EICHER, E. & ZAMAN, M. (1984) A study of health and productivity of traditionally managed cattle in Zambia. *Preventive Veterinary Medicine,* 2, 633-653.

PHIRI, I. K., DORNY, P., GABRIEL, S., WILLINGHAM, A. L., 3RD, SPEYBROECK, N. & VERCRUYSSE, J. (2002) The prevalence of porcine cysticercosis in Eastern and Southern provinces of Zambia. *Vet Parasitol,* 108, 31-9.

PHIRI, I. K., NGOWI, H., AFONSO, S., MATENGA, E., BOA, M., MUKARATIRWA, S., GITHIGIA, S., SAIMO, M., SIKASUNGE, C., MAINGI, N., LUBEGA, G. W., KASSUKU, A., MICHAEL, L., SIZIYA, S., KRECEK, R. C., NOORMAHOMED, E., VILHENA, M., DORNY, P. & WILLINGHAM, A. L., 3RD (2003) The emergence of Taenia solium cysticercosis in Eastern and Southern Africa as a serious agricultural problem and public health risk. *Acta Trop,* 87, 13-23.

PICOZZI, K., CARRINGTON, M. & WELBURN, S. C. (2008) A multiplex PCR that discriminates between Trypanosoma brucei brucei and zoonotic T. b. rhodesiense. *Exp Parasitol,* 118, 41-6.

POPE, A. (2005) Luangwa Safari Association Tourism Study. Citeseer.

PREIS, T., MOAT, H. S., STANLEY, H. E. & BISHOP, S. R. (2012) Quantifying the Advantage of Looking Forward. *Scientific Reports,* 2.

PRESCOTT-ALLEN, R. (2001) *The wellbeing of nations*, Island Press

PROJECT-LUANGWA. Education in Zambia [online]. Available from: http://www.projectluangwa.org/education [August 2015]

PROMED-MAIL (2013a) African swine fever Zambia: (Lusaka) OIE, PROMED-MAIL, <**http://www.promedmail.org/**. 20 November 2013

PROMED-MAIL (2013b) Anthrax Zambia: Western, Human, Bovine, PROMED-MAIL, <**http://www.promedmail.org/**. 22 November 2013

PROMED-MAIL (2013c) Cyanide poisoning, elephants-Zimbabwe: Hwange National Park, PROMED-MAIL, <http://www.promedmail.org/>. 3 October 2013

PROMED-MAIL (2013d) Heartwater-Chad: (Guera) Camelidae, OIE, PROMED-MAIL, <http://www.promedmail.org/>. 31 December 2013

PROMED-MAIL (2014a) African swine fever Zambia: (LS) RFI, PROMED-MAIL, <**http://www.promedmail.org/**. 21 October 2014

PROMED-MAIL (2014b) Malaria, Plasmodium knowlesi, human - Malaysia, <http://www.promedmail.org/>. 3 November 2014

PROMED-MAIL (2014c) Malaria: World Malaria Report, Challenges of drug resistance, <http://www.promedmail.org/>. 18 December 2014

PROMED-MAIL (2014d) Transboundary disease transmission-USA: risk from wildlife trade, <http://www.promedmail.org/>. 01 September 2014

PROMED-MAIL (2014e) Trypanosomiasis, African - Zambia: (Lusaka), <http://www.promedmail.org/>. 22 April 2014

PROMED-MAIL (2015a) Anaplasmosis-China: (HL) new species, humans, goats, tick-borne, PROMED-MAIL, <http://www.promedmail.org/>. 4 April 2015

PROMED-MAIL (2015b) Anthrax Zambia: Western, Human, Bovine, PROMED-MAIL, <**http://www.promedmail.org/**. 10 April 2015

PROMED-MAIL (2015c) Counterfeit medications-worldwide, <http://www.promedmail.org/>. 27 April 2015

PROMED-MAIL (2015d) Foot and mouth disease Zambia: Western, bovine, serotype pending, OIE, PROMED-MAIL, <**http://www.promedmail.org/**. 28 October 2015

PROMED-MAIL (2015e) Lumpy skin disease, bovine: Kuwait (Al Jahrah), Zambia (Muchinga), PROMED-MAIL, <**http://www.promedmail.org/**. 5 February 2015

PROMED-MAIL (2015f) Peste des petits ruminants Zambia: Caprine, OIE, PROMED-MAIL, <**http://www.promedmail.org/**. 7 July 2015

PROMED-MAIL (2015g) Plague-Zambia: (Eastern Province) bubonic, suspected, <http://www.promedmail.org/>. 16 April 2015

PROMED-MAIL (2015h) Rickettsia felis, mosquito-borne, susp., PROMED-MAIL, <http://www.promedmail.org/>. 21 June 2015

PROMED-MAIL (2015i) Trypanosomiasis, African-Canada ex Zambia, <http://www.promedmail.org/>. 4 September 2015

PROMED-MAIL (2016a) Bovine tuberculosis, wildlife - South Africa: (Kruger Park) African wild dog, <http://www.promedmail.org/>. 21 March 2016

PROMED-MAIL (2016b) Trypanosomiasis, African-USA: (Maryland) ex Botswana and Zambia, <http://www.promedmail.org/>. 22 December 2016

PROMED-MAIL (2017a) Hendra Virus- Australia: (Eastern) increased risk, human intrusion on fruit bat habitats, <http://www.promedmail.org/>. 20 August 2017

PROMED-MAIL (2017b) Trypanosomiasis-Germany ex Zambia, <http://www.promedmail.org/>. 16 August 2017

PUTT, S. N. H., SHAW, A. P. M., WOODS, A. J., L.TYLER & JAMES, A. D. (1988) Veterinary epidemiology and economics in Africa- A manual for use in the design and appraisal of livestock health policy.

RANDOLPH, T., SCHELLING, E., GRACE, D., NICHOLSON, C. F., LEROY, J., COLE, D., DEMMENT, M., OMORE, A., ZINSSTAG, J. & RUEL, M. (2007) Role of livestock in human nutrition and health for poverty reduction in developing countries. *Journal of animal science,* 85, 2788-2800.

RAOULT, D., FOURNIER, P. E., FENOLLAR, F., JENSENIUS, M., PRIOE, T., DE PINA, J. J., CARUSO, G., JONES, N., LAFERL, H., ROSENBLATT, J. E. & MARRIE, T. J. (2001) Rickettsia africae, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med,* 344, 1504-10.

RATH, T. & HARTER, J. K. (2010) *Wellbeing: The five essential elements*, Gallup Press

REDEFINING PROGRESS. (2016) Genuine progress indicator (GPI) [online]. Available from: http://rprogress.org/sustainability_indicators/genuine_progress_indicator.htm

REID, R. S., KRUSKA, R. L., DEICHMANN, U., THORNTON, P. K. & LEAK, S. G. (2000) Human population growth and the extinction of the tsetse fly. *Agriculture, Ecosystems & Environment,* 77, 227-236.

RIDDER, B. (2008) Questioning the ecosystem services argument for biodiversity conservation. *Biodiversity and conservation,* 17, 781-790.

RODGERS, J., JONES, A., GIBAUD, S. P., BRADLEY, B., MCCABE, C., BARRETT, M. P., GETTINBY, G. & KENNEDY, P. G. (2011) Melarsoprol cyclodextrin inclusion complexes as promising oral candidates for the treatment of human African trypanosomiasis. *PLOS Neglected Tropical Diseases,* 5, e1308.

ROSA, C. T., PAZZI, P., NAGEL, S., MCCLURE, V., CHRISTIE, J., TROSKIE, M. & DVIR, E. (2014) Theileriosis in six dogs in South Africa and its potential clinical significance. *J S Afr Vet Assoc,* 85, 1114.

ROTTBECK, R., NSHIMIYIMANA, J. F., TUGIRIMANA, P., DULL, U. E., SATTLER, J., HATEGEKIMANA, J. C., HITAYEZU, J., BRUCKMAIER, I., BORCHERT, M., GAHUTU, J. B., DIECKMANN, S., HARMS, G., MOCKENHAUPT, F. P. & IGNATIUS, R. (2013) High prevalence of cysticercosis in people with epilepsy in southern Rwanda. *PLoS Negl Trop Dis,* 7, e2558.

RUSHTON, J. (2009) *The economics of animal health and production,* Wallingford, Oxfordshire, UK ; Cambridge, MA, CABI

SAGAN, L. A. (1987) The health of nations: true causes of sickness and well-being.

SALKELD, D. J., PADGETT, K. A. & JONES, J. H. (2013) A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecology Letters,* 16, 679-686.

SAMUI, K. L., NAMBOTA, A. M., MWEENE, A. S. & ONUMA, M. (1996) African swine fever in Zambia: potential financial and production consequences for the commercial sector. *Jpn J Vet Res,* 44, 119-24.

SANDIFER, P. A., SUTTON-GRIER, A. E. & WARD, B. P. (2015) Exploring connections among nature, biodiversity, ecosystem services, and human health and well-being: Opportunities to enhance health and biodiversity conservation. *Ecosystem Services,* 12, 1-15.

SCASTA, J. D. (2015) Fire and parasites: an under-recognized form of anthropogenic land use change and mechanism of disease exposure. *Ecohealth*, 1-6.

SCHLAGENHAUF, P. & PETERSEN, E. (2009) Antimalaria drug resistance: the mono-combi-counterfeit triangle. *Expert review of anti-infective therapy,* 7, 1039-1042.

SCHOULS, L. M., VAN DE POL, I., RIJPKEMA, S. G. & SCHOT, C. S. (1999) Detection and identification of Ehrlichia, Borrelia burgdorferi sensu lato, and Bartonella species in Dutch Ixodes ricinus ticks. *J Clin Microbiol,* 37, 2215-22.

SCHUENEMANN, V. J., SINGH, P., MENDUM, T. A., KRAUSE-KYORA, B., JÃ¤GER, G. N., BOS, K. I., HERBIG, A., ECONOMOU, C., BENJAK, A., BUSSO, P., NEBEL, A., BOLDSEN, J. L., KJELLSTRÃ¶M, A., WU, H., STEWART, G. R., TAYLOR, G. M., BAUER, P., LEE, O. Y.-C., WU, H. H. T., MINNIKIN, D. E., BESRA, G. S., TUCKER, K., ROFFEY, S., SOW, S. O., COLE, S. T., NIESELT, K. & KRAUSE, J. (2013) Genome-Wide Comparison of Medieval and Modern *Mycobacterium leprae*. *Science,* 341, 179-183.

SCHUURMAN, H. J. (1983) The serological response of adult cattle to vaccination with reduced dose Brucella abortus S19, a trial under Zambian conditions. *Vet Q,* 5, 94-6.

SCHWABE, C. W. (1964) Veterinary medicine and human health. *Veterinary medicine and human health.*

SCOTCH, M., ODOFIN, L. & RABINOWITZ, P. (2009) Linkages between animal and human health sentinel data. *BMC veterinary research,* 5, 1.

SEEBREGTS, C. J., ZWARENSTEIN, M., MATHEWS, C., FAIRALL, L., FLISHER, A. J., SEEBREGTS, C., MUKOMA, W. & KLEPP, K.-I. (2009) Handheld computers for survey and trial data collection in resource-poor settings: Development and evaluation of PDACT, a Palmâ„¢ Pilot interviewing system. *International journal of medical informatics,* 78, 721-731.

SEID, M. A., YOSEPH, L. W., BEFEKADU, U. W., MUHAMMED, A. & FIKRE, T. Z. (2016) Communication for the development of pastoralism. *Rev Sci Tech,* 35, 639-648.

SEKELI, P. M. & PHIRI, M. (2002) State of Forest and Tree Genetic Resources in Zambia., FOREST RESOURCES DEVELOPMENT SERVICE, F. R. D.,

SEO, M. K., BAKER, P. & NGO, K. N. (2014) Cost-effectiveness analysis of vaccinating children in Malawi with RTS,S vaccines in comparison with long-lasting insecticide-treated nets. *Malar J,* 13, 66.

SHARGIE, E. B., GEBRE, T., NGONDI, J., GRAVES, P. M., MOSHER, A. W., EMERSON, P. M., EJIGSEMAHU, Y., ENDESHAW, T., OLANA, D., WELDEMESKEL, A., TEFERRA, A., TADESSE, Z., TILAHUN, A., YOHANNES, G. & RICHARDS, F. O., JR. (2008) Malaria prevalence and mosquito net coverage in Oromia and SNNPR regions of Ethiopia. *BMC Public Health,* 8, 321.

SHIFF, C. J., STOYANOV, C., CHOOBWE, C., KAMANGA, A. & MUKONKA, V. M. (2013) Measuring malaria by passive case detection: a new perspective based on Zambian experience. *Malaria journal,* 12, 120.

SIKASUNGE, C. S., PHIRI, I. K., PHIRI, A. M., DORNY, P., SIZIYA, S. & WILLINGHAM, A. L., 3RD (2007) Risk factors associated with porcine cysticercosis in selected districts of Eastern and Southern provinces of Zambia. *Vet Parasitol,* 143, 59-66.

SIKASUNGE, C. S., PHIRI, I. K., PHIRI, A. M., SIZIYA, S., DORNY, P. & WILLINGHAM, A. L., 3RD (2008) Prevalence of Taenia solium porcine cysticercosis in the Eastern, Southern and Western provinces of Zambia. *Vet J,* 176, 240-4.

SILBERMAYR, K., LI, F., SOUDRÃ©, A., MÃ¼LLER, S. & SÃ¶LKNER, J. (2013) A novel qPCR assay for the detection of African Animal Trypanosomosis in trypanotolerant and trypanosusceptible cattle breeds. *PLoS Negl Trop Dis,* 7, e2345.

SIMARRO, P. P., CECCHI, G., FRANCO, J. R., PAONE, M., DIARRA, A., RUIZ-POSTIGO, J. A., FÈVRE, E. M., MATTIOLI, R. C. & JANNIN, J. G. (2012) Estimating and mapping the population at risk of sleeping sickness. *PLOS Neglected Tropical Diseases,* 6, e1859.

SIMONSEN, P. E., ONAPA, A. W. & ASIO, S. M. (2011) Mansonella perstans filariasis in Africa. *Acta Trop,* 120 Suppl 1, S109-20.

SIMUKOKO, H., MARCOTTY, T., PHIRI, I., GEYSEN, D., VERCRUYSSE, J. & VAN DEN BOSSCHE, P. (2007a) The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Veterinary parasitology,* 147, 231-238.

SIMUKOKO, H., MARCOTTY, T., PHIRI, I., VERCRUYSSE, J. & VAN DEN BOSSCHE, P. (2007b) Heterogeneity in the trypanosomosis incidence in Zebu cattle of different ages and sex on the plateau of eastern Zambia. *Acta Trop,* 103, 98-101.

SIMULUNDU, E., CHAMBARO, H. M., SINKALA, Y., KAJIHARA, M., OGAWA, H., MORI, A., NDEBE, J., DAUTU, G., MATAA, L., LUBABA, C. H., SIMUNTALA, C., FANDAMU, P., SIMUUNZA, M., PANDEY, G. S., SAMUI, K. L., MISINZO, G., TAKADA, A. & MWEENE, A. S. (2017) Co-circulation of multiple genotypes of African swine fever viruses among domestic pigs in Zambia (2013-2015). *Transbound Emerg Dis*.

SIMUUNZA, M., WEIR, W., COURCIER, E., TAIT, A. & SHIELS, B. (2011) Epidemiological analysis of tick-borne diseases in Zambia. *Vet Parasitol,* 175, 331-42.

SINDATO, C., KIBONA, S., NKYA, G., MBILU, T., MANGA, C., KABOYA, J. & RAWILLE, F. (2008) Challenges in the diagnosis and management of sleeping sickness in Tanzania: a case report. *Tanzania Journal of Health Research,* 10, 177-181.

SINYANGWE, L., DELESPAUX, V., BRANDT, J., GEERTS, S., MUBANGA, J., MACHILA, N., HOLMES, P. & EISLER, M. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary parasitology,* 119, 125-135.

SIRAJ, A., SANTOS-VEGA, M., BOUMA, M., YADETA, D., CARRASCAL, D. R. & PASCUAL, M. (2014) Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. *Science,* 343, 1154-1158.

SIWILA, J., PHIRI, I. G., ENEMARK, H. L., NCHITO, M. & OLSEN, A. (2010) Intestinal helminths and protozoa in children in pre-schools in Kafue district, Zambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 104, 122-128.

SIZIYA, S., MUULA, A. S., BESA, C., BABANIYI, O., SONGOLO, P., KANKIZA, N. & RUDATSIKIRA, E. (2013) Cannabis use and its socio-demographic correlates among in-school adolescents in Zambia. *Italian journal of pediatrics,* 39, 13.

SIZIYA, S., MUULA, A. S. & RUDATSIKIRA, E. (2011) Self-reported poor oral hygiene among in-school adolescents in Zambia. *BMC Res Notes,* 4, 255.

SMIRNOVA, E. A., VASIN, A. V., SANDYBAEV, N. T., KLOTCHENKO, S. A., PLOTNIKOVA, M. A., CHERVYAKOVA, O. V., SANSYZBAY, A. R. & KISELEV, O. I. (2013) Current methods of human and animal Brucellosis diagnostics.

SMITH, L. M., CASE, J. L., SMITH, H. M., HARWELL, L. C. & SUMMERS, J. (2013) Relating ecoystem services to domains of human well-being: Foundation for a US index. *Ecological Indicators,* 28, 79-90.

SONGA, E. B., HAMERS, R., RICKMAN, R., NANTULYA, V., MULLA, A. & MAGNUS, E. (1991) Evidence for widespread asymptomatic Trypanosoma rhodesiense human infection in the Luangwa Valley (Zambia). *Tropical medicine and parasitology: official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ),* 42, 389-393.

SPENCE, P. J., BRUGAT, T. & LANGHORNE, J. (2015) Mosquitoes reset malaria parasites. *PLoS pathogens,* 11, e1004987.

STEVENS, P. (2004) Diseases of poverty and the 10/90 gap. *International Policy Network,* *http://who. int/intellectualproperty/submissions/InternationalPolicyNetwork. pdf.*

STONE, L., RAJAGOPALAN, B., BHASIN, H. & LOYA, Y. (1999) Mass Coral Reef Bleaching: A Recent Outcome of Increased El Niño Activity? *Ecology Letters,* 2, 325-330.

SUZUKI, M. (2011) " Would They be Lazier or Work Harder Given Free Money?": The Namibia BIG Pilot Project and the Possibility of Basic Income as a Strategy of Social Cooperation. *Journal of Political Science & Sociology*.

SWALLOW, B. M. (2000) Impacts of trypanosomiasis on African agriculture. *PAAT Technical and ScientiÂ® c Series, FAO.* Citeseer.

TANGA, M. C. & NGUNDU, W. (2010) Ecological transition from natural forest to tea plantations: effect on the dynamics of malaria vectors in the highlands of Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 104, 659-668.

TEWKSBURY, J. J. & ROGERS, H. S. (2014) An animal-rich future. *Science,* 345, 400.

THE ECONOMIST. (2017) 'Big-Mac' index [online]. Available from: https://www.economist.com/blogs/graphicdetail/2017/07/daily-chart-7

THOMAS, L. F., DE GLANVILLE, W. A., COOK, E. A. & FÈVRE, E. M. (2013) The spatial ecology of free-ranging domestic pigs (Sus scrofa) in western Kenya. *BMC Vet Res,* 9, 46.

THUMBI, S. M., DE, C. B. B. M., POOLE, E. J., KIARA, H., TOYE, P., NDILA, M., CONRADIE, I., JENNINGS, A., HANDEL, I. G., COETZER, J. A., HANOTTE, O. & WOOLHOUSE, M. E. (2013) Parasite co-infections show synergistic and antagonistic interactions on growth performance of East African zebu cattle under one year. *Parasitology,* 140, 1789-98.

THUMBI, S. M., DE CLARE BRONSVOORT, B. M., POOLE, E. J., KIARA, H., TOYE, P. G., MBOLE-KARIUKI, M. N., CONRADIE, I., JENNINGS, A., HANDEL, I. G. & COETZER, J. A. W. (2014) Parasite co-infections and their impact on survival of indigenous cattle. *PLoS One,* 9, e76324.

TINKLER, L. & HICKS, S. (2011) Measuring subjective well-being. *London: Office for National Statistics*, 443-455.

TORGERSON, P. R. & MACPHERSON, C. N. (2011) The socioeconomic burden of parasitic zoonoses: global trends. *Vet Parasitol,* 182, 79-95.

TORR, S. J., MANGWIRO, T. N. & HALL, D. R. (2006) The effects of host physiology on the attraction of tsetse (Diptera: Glossinidae) and Stomoxys (Diptera: Muscidae) to cattle. *Bull Entomol Res,* 96, 71-84.

TREERUTKUARKUL, A. (2016) Bringing an Innovative Health Surveillance System to Communities [online]. South East Asia One Health University Network (SEAOHUN). Available from: https://medium.com/one-health-workforce/bringing-an-innovative-health-surveillance-system-to-communities-de3828cda296#.69l05q89x

TSHALA-KATUMBAY, D., MUMBA, N., OKITUNDU, L., KAZADI, K., BANEA, M., TYLLESKAR, T., BOIVIN, M. & MUYEMBE-TAMFUM, J. J. (2013) Cassava food toxins, konzo disease, and neurodegeneration in sub-Sahara Africans. *Neurology,* 80, 949-51.

UN (2011a) Happiness : towards a holistic approach to development : resolution / adopted by the General Assembly. IN QUALITY OF LIFE, HUMAN DEVELOPMENT & INFORMATION EXCHANGE (Eds.).

UN (2011b) Happiness should have greater role in development policy – UN Member States.

UN. (2015) Sustainable development goals [online]. Available from: http://www.un.org/sustainabledevelopment/sustainable-development-goals/

UNDP. (2016) Human development index (HDI) [online]. Available from: http://hdr.undp.org/en/content/human-development-index-hdi

UNSTATS (2015) Population and Vital Statistics Report, VALBRUN, V. (2017) Equity vs. Equality: Eliminating Opportunity Gaps in Education [online]. Available from: http://www.theinclusionsolution.me/equity-vs-equality-eliminating-opportunity-gaps-education/

VAN DEN BOSSCHE, P. (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomosis in southern Africa. *International journal for parasitology,* 31, 592-598.

VAN DEN BOSSCHE, P., CHITANGA, S., MASUMU, J., MARCOTTY, T. & DELESPAUX, V. (2011) Virulence in Trypanosoma congolense Savannah subgroup. A comparison between strains and transmission cycles. *Parasite immunology,* 33, 456-460.

VAN DEN BOSSCHE, P. & DE DEKEN, R. (2002) Seasonal variations in the distribution and abundance of the tsetse fly, Glossina morsitans morsitans in eastern Zambia. *Med Vet Entomol,* 16, 170-6.

VAN DEN BOSSCHE, P., DE LA ROCQUE, S. P., HENDRICKX, G. & BOUYER, J. R. M. (2010) A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. *Trends in parasitology,* 26, 236-243.

VAN DEN BOSSCHE, P. & DELESPAUX, V. (2011) Options for the control of tsetse-transmitted livestock trypanosomosis. An epidemiological perspective. *Veterinary parasitology,* 181, 37-42.

VAN DEN BOSSCHE, P., DORAN, M. & CONNOR, R. (2000) An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta tropica,* 75, 247-258.

VAN DEN ENDEN, E. (2012) African Trypanosomiasis- Clinical [online]. Antwerp, Institute of Tropical Medicine (ITM). Available from: http://itg.content-e.eu/Generated/pubx/173/african_trypanosomiasis/clinical.htm [May 2015]

VLAHAKIS, P. A., CHITANGA, S., SIMUUNZA, M. C., SIMULUNDU, E., QIU, Y., CHANGULA, K., CHAMBARO, H. M., KAJIHARA, M., NAKAO, R., TAKADA, A. & MWEENE, A. S. (2017) Molecular detection and characterization of zoonotic Anaplasma species in domestic dogs in Lusaka, Zambia. *Ticks Tick Borne Dis*.

VON WISSMANN, B., MACHILA, N., PICOZZI, K., FEVRE, E. M., DE, C. B. B. M., HANDEL, I. G. & WELBURN, S. C. (2011) Factors associated with acquisition of human infective and animal infective trypanosome infections in domestic livestock in Western Kenya. *PLoS Negl Trop Dis,* 5, e941.

WALKER, B. H. (1992) Biodiversity and ecological redundancy. *Conservation biology,* 6, 18-23.

WASSER, S., BROWN, L., MAILAND, C., MONDOL, S., CLARK, W., LAURIE, C. & WEIR, B. (2015) Genetic assignment of large seizures of elephant ivory reveals Africaâ€™s major poaching hotspots. *Science*, aaa2457.

WATKINS, K. (2007) Human development report 2007/2008: Fighting climate change: Human solidarity in a divided world.

WEINSTEIN, M. C. & STASON, W. B. (1977) Foundations of cost-effectiveness analysis for health and medical practices. *New England journal of medicine,* 296, 716-721.

WELBURN, S. C., BEANGE, I., DUCROTOY, M. J. & OKELLO, A. L. (2015) The neglected zoonoses--the case for integrated control and advocacy. *Clin Microbiol Infect,* 21, 433-43.

WELBURN, S. C., FEVRE, E. M., COLEMAN, P. G., ODIIT, M. & MAUDLIN, I. (2001) Sleeping sickness: a tale of two diseases. *Trends Parasitol,* 17, 19-24.

WELBURN, S. C. & MAUDLIN, I. (1987) A simple in vitro method for infecting tsetse with trypanosomes. *Ann Trop Med Parasitol,* 81, 453-5.

WELBURN, S. C. & MAUDLIN, I. (2012) Priorities for the elimination of sleeping sickness. *Adv Parasitol,* 79, 299-337.

WHITEHEAD, S. J. & ALI, S. (2010) Health outcomes in economic evaluation: the QALY and utilities. *British Medical Bulletin,* 96, 5-21.

WHO. (1948) Constitution of WHO: principles [online]. Available from: http://www.who.int/about/mission/en/

WHO (2003) World Health Survey Results, <http://who.int/healthinfo/survey/whsresults/en/index1.html>.

WHO (2011) The control of neglected zoonotic diseases: community based interventions for NZDs prevention and control: report of the third conference organized with ICONZ, DFID-RiU, SOS, EU, TDR and FAO with the participation of ILRI and OIE: 23-24 November 2010, WHO Heaquarters, Geneva, Switzerland,

WHO. (2012a) Human African trypanosomiasis (sleeping sickness) [online]. Available from: http://www.who.int/mediacentre/factsheets/fs259/en/

WHO (2012b) Malaria rapid diagnostic test performance: Summary results of WHO product testing of malaria RDTs: Round 1-4 (2008-2012),

WHO. (2012c) Metrics: Disability-Adjusted Life Year (DALY) [online]. Available from: http://www.who.int/healthinfo/global_burden_disease/metrics_daly/en/

WHO (2014) Global Status Report on noncommunicable diseases 2014, <http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua=1>.

WHO (2015) Connecting Global Priorities: Biodiversity and Human Health. World Health Organization and Secretariat of the Convention on Biological Diversity.

WHO (2016) International Health Regulations (2005), <http://apps.who.int/iris/bitstream/10665/246107/1/9789241580496-eng.pdf?ua=1>.

WHO. (2017a) Health systems: equity [online]. Available from: http://www.who.int/healthsystems/topics/equity/en/

WHO. (2017b) UHC2030: Our mission [online]. Available from: https://www.uhc2030.org/our-mission/

WHO, DFID, FAO & OIE (2006) The control of neglected zoonotic diseases : a route to poverty alleviation : report of a joint WHO/DFID-AHP meeting, 20 and 21 September 2005, WHO Headquarters, Geneva, with the participation of FAO and OIE, < http://www.who.int/zoonoses/Report_Sept06.pdf>.

WHO (NTD). Neglected tropical diseases [online]. Available from: http://www.who.int/neglected_diseases/diseases/en/ [6th July 2015]

WHO (NZD). Neglected zoonotic diseases [online]. Available from: http://www.who.int/neglected_diseases/diseases/zoonoses/en/ [6th July 2015]

WHO (SDH). The social determinants of health (SDH) [online]. Available from: http://www.who.int/social_determinants/en/

WIETHOELTER, A. K., BELTRAN-ALCRUDO, D., KOCK, R. & MOR, S. M. (2015) Global trends in infectious diseases at the wildlife-livestock interface. *Proc Natl Acad Sci U S A*.

WILBY, K. J., LAU, T. T., GILCHRIST, S. E. & ENSOM, M. H. (2012) Mosquirix (RTS, S): a novel vaccine for the prevention of Plasmodium falciparum malaria. *Annals of Pharmacotherapy,* 46, 384-393.

WILKINSON, P. J., PEGRAM, R. G., PERRY, B. D., LEMCHE, J. & SCHELS, H. F. (1988) The distribution of African swine fever virus isolated from Ornithodoros moubata in Zambia. *Epidemiol Infect,* 101, 547-64.

WILKINSON, R. G. & PICKETT, K. E. (2006) Income inequality and population health: a review and explanation of the evidence. *Social science & medicine,* 62, 1768-1784.

WOOLHOUSE, M. E., MCNAMARA, J. J., HARGROVE, J. W. & BEALBY, K. A. (1996) Distribution and abundance of trypanosome (subgenus Nannomonas) infections of the tsetse fly Glossina pallidipes in southern Africa. *Mol Ecol,* 5, 11-18.

WOOLHOUSE, M. E., THUMBI, S. M., JENNINGS, A., CHASE-TOPPING, M., CALLABY, R., KIARA, H., OOSTHUIZEN, M. C., MBOLE-KARIUKI, M. N., CONRADIE, I., HANDEL, I. G., POOLE, E. J., NJIIRI, E., COLLINS, N. E., MURRAY, G., TAPIO, M., AUGUET, O. T., WEIR, W., MORRISON, W. I., KRUUK, L. E., BRONSVOORT, B. M., HANOTTE, O., COETZER, K. & TOYE,

P. G. (2015) Co-infections determine patterns of mortality in a population exposed to parasite infection. *Sci Adv,* 1, e1400026.

WORLD BANK (2010) People, pathogens and our planet, Volume 1: Towards a One Health approach for controlling zoonotic diseases. Washington DC,. <http://siteresources.worldbank.org/INTARD/Resources/PPP_Web.pdf>.

WORLD BANK. (2012) World Data Bank [online]. Available from: http://databank.worldbank.org/data/home.aspx [July 2012]

WORLD BANK (2012) People, pathogens and our planet Volume 2: The economics of one health. WASHINGTON DC, <http://documents.worldbank.org/curated/en/612341468147856529/People-pathogens-and-our-planet-the-economics-of-one-health >.

WU, W., JIA, F., WANG, W. & HUANG, Y. (2013) Antiparasitic treatment of cerebral cysticercosis: lessons and experiences from China. *Parasitol Res,* 112, 2879-90.

WURAPA, F., BULSARA, M., CHEN, M. & WYATT, G. (1984a) The public health importance of African trypanosomiasis in north east Zambia. *Tropical and geographical medicine,* 36, 329-333.

WURAPA, F., DUKES, P., NJELESANI, E. & BOATIN, B. (1984b) A healthy carrier of Trypanosoma rhodesiense: a case report. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 78, 349-350.

YEBOAH-ANTWI, K., PILINGANA, P., MACLEOD, W. B., SEMRAU, K., SIAZEELE, K., KALESHA, P., HAMAINZA, B., SEIDENBERG, P., MAZIMBA, A. & SABIN, L. (2010) Community case management of fever due to malaria and pneumonia in children under five in Zambia: a cluster randomized controlled trial. *PLoS medicine,* 7, e1000340.

YEPES, M., MAURER, J., VISWANATHAN, B., GEDEON, J. & BOVET, P. (2016) Potential Reach of mHealth Versus Traditional Mass Media for Prevention of Chronic Diseases: Evidence From a Nationally Representative Survey in a Middle-Income Country in Africa. *J Med Internet Res,* 18, e114.

YILMAZ, B., PORTUGAL, S., TRAN, T. M., GOZZELINO, R., RAMOS, S., GOMES, J., REGALADO, A., COWAN, P. J., DÂ€™APICE, A. J. & CHONG, A. S. (2014) Gut microbiota elicits a protective immune response against malaria transmission. *Cell,* 159, 1277-1289.

YUKICH, J. O., BENNETT, A., ALBERTINI, A., INCARDONA, S., MOONGA, H., CHISHA, Z., HAMAINZA, B., MILLER, J. M., KEATING, J. & EISELE, T. P. (2012) Reductions in artemisinin-based combination therapy consumption after the nationwide scale up of routine malaria rapid diagnostic testing in Zambia. *The American journal of tropical medicine and hygiene,* 87, 437-446.

YUSUFMIA, S. B., COLLINS, N. E., NKUNA, R., TROSKIE, M., VAN DEN BOSSCHE, P. & PENZHORN, B. L. (2010) Occurrence of Theileria parva and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. *J S Afr Vet Assoc,* 81, 45-9.

ZAWA (2011) Lupande game management area general management plan 2011-2020.

ZINSSTAG, J., SCHELLING, E., ROTH, F., BONFOH, B., DE SAVIGNY, D. & TANNER, M. (2007a) Human benefits of animal interventions for zoonosis control. *Emerging infectious diseases,* 13, 527.

ZINSSTAG, J., SCHELLING, E., ROTH, F., BONFOH, B., SAVIGNY, D. D. & TANNER, M. (2007b) Human health and societal benefits of interventions in animals. *Proceedings of the 12th International Conference of the Association of Institutions for Tropical Veterinary Medicine (AITVM), Montpellier, France, 20-22 August, 2007. Does control of animal infectious risks offer a new international perspective?* , Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD).

ZINSSTAG, J. & TANNER, M. (2008) One health: the potential of closer cooperation between human and animal health in Africa. *Ethiop J Health Dev,* 22, 105-108.

ZWEYGARTH, E., JOSEMANS, A. I., SPICKETT, A. M., STEYN, H. C., PUTTERILL, J., TROSKIE, P. C., MTSHALI, M. S., BELL-SAKYI, L., SHKAP, V., FISH, L., KOCAN, K. M. & BLOUIN, E. F. (2006) In vitro cultivation of a south African isolate of an Anaplasma sp. in tick cell cultures. *Onderstepoort J Vet Res,* 73, 251-5.